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Improved stability of methane-producing anaerobic biological reactors through novel use of ion-exchange fibers

Yu Tian
Lehigh University

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Yu Tian

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Date

Dr. Derick G. Brown, Dissertation Director
Professor of Civil and Environmental Engineering

Date of Acceptance

Dr. Arup K. SenGupta, Dissertation Director
Professor of Civil and Environmental Engineering

Committee Members:

Dr. John T. Fox
Dissertation Committee Chairperson
Professor of Civil and Environmental Engineering

Dr. Kristen L. Jellison
Dissertation Committee member
Professor of Civil and Environmental Engineering

Dr. Bryan W. Berger
Dissertation External Committee member
Professor of Bioengineering and Chemical and Biomolecular Engineering

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ABSTRACT

The anaerobic digestion (AD) process uses a series of microbial reactions to convert organic compounds to a methane-rich biogas, and engineered application of this process allows energy recovery from industrial organic waste streams. Poor stability and susceptibility to failure, however, have greatly hindered more frequent application of AD for treatment of industrial wastes. The concern with process stability is particularly true where reactor pH fluctuates due to variations in organic loading. During the organic overloading, the huge difference of growth rate between acetogenic bacteria and methanogenic archaea could induce the accumulation of volatile fatty acids. The accumulated volatile fatty acids will drop pH and the lower pH can cause severe stress conditions on the methanogenic archaea within the reactors. And also heavy metals have been reported to be one of the major causes of digester upset or failure. In present, there are two major ways to alleviate heavy metal toxicity. One is the addition of chemicals, such as sulfide, EDTA and NTA. However, these chemicals are also toxic to anaerobic digester if they are excessive used. The other is the addition of solid surface including activated carbon, kaolin, needle-punctured fabric. These solid materials can be inert or active. Here, this dissertation demonstrated that the iminodiacetate-functionalized ion-exchange fiber (IXF) Fiban X-1 is able to stabilize AD by buffering pH fluctuations and moderating shock-loads of dissolved toxic metals and the anion ion exchange fiber Fiban A-1 was also used to alleviate chromate toxicity successfully. Compared to other active material, it has

high regeneration rate, reaction rate, selectivity, capacity and very stable to high alkaline solution. Results provide positive data indicating that IXFs can be used to passively stabilize engineered anaerobic reactors against organic overloading events. Reactors with IXF always have higher pH, methane generation and lower COD concentration than the reactor without IXF during organic overloading events. Also, the relationships among the methane generation, pH and COD with all the data from organic overloading experiments were studied. The result showed that the performance of reactors is proportional to pH. But unexpectedly COD or VFA concentration doesn't have a clear relationship with normalized methane generation rate. For experiments of the addition of heavy metals, the effluent metal concentration and methane generation proved that the ion exchange capacity helped reactors survived from heavy metals' toxicity. However, ion exchange capacity exhibited different importance in nickel and copper addition experiment, when they are compared with methane generation of the reactor with glass-fiber that doesn't have ion exchange capacity. In addition, after exposure, reactors showed a significant pH drop and all of failed reactors had a low pH around 5.

1. INTRODUCTION AND BACKGROUND

1.1 Anaerobic biological processes are able to convert organic waste streams to a methane-rich biogas that can be used as a renewable biofuel

Sustainable energy generation is a requisite goal for the long-term needs of society. An appropriate approach to achieving this goal is the development of a diverse portfolio of technologies that make use of renewable energy sources. One relatively untapped energy source is organic-rich, high-strength waste streams from the agricultural, food processing, beverage and chemical industries. The main technology for converting organic waste streams into usable energy is via methane-producing anaerobic biological reactors (MPABRs), such as anaerobic digestion, where organics are converted to a methane-rich biogas (Figures 1.1). It does not require any external supply of oxygen (as with aerobic treatment) and produces only a small amount of sludge. Anaerobic digestion has been used for many years in the treatment of sludge resulting from aerobic wastewater treatment; however, only a fraction of the overall available energy is recovered and aerobic plants remain energy negative. It has been estimated that if all wastewater treatment plants in the United States utilized MPABRs for wastewater treatment and produced electricity from the resulting methane biogas, it would provide anywhere from 3,300 to 4,940 GWh/yr.[1] This is enough electricity to

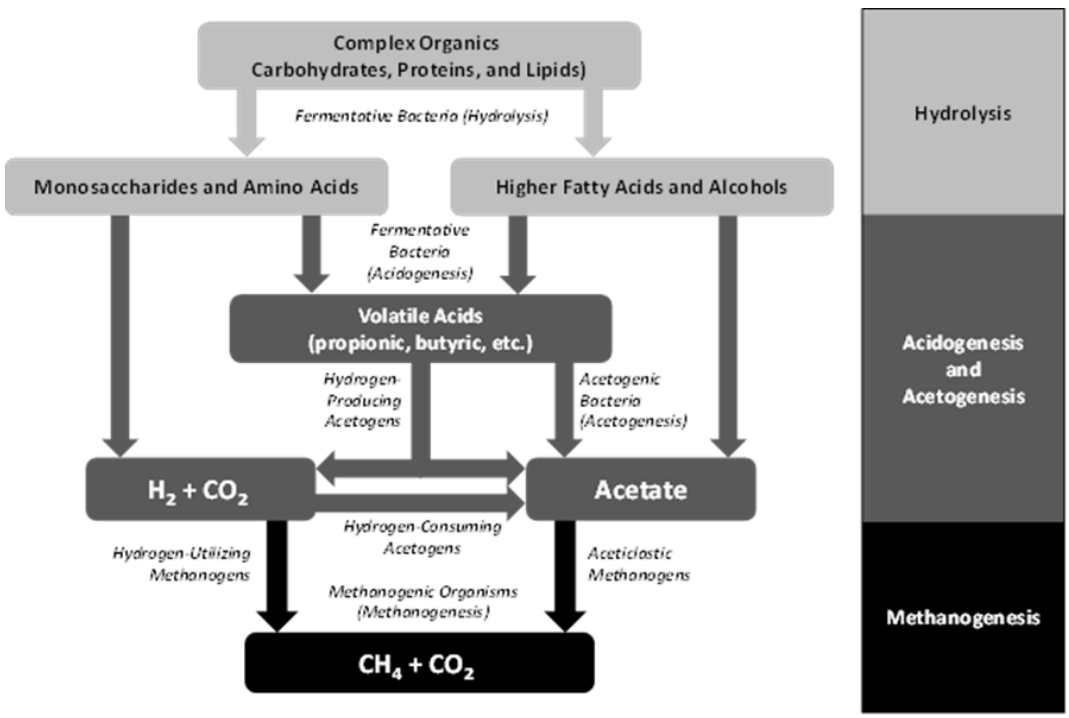

Figure 1.1 Metabolic pathways and microbial groups involved in the production of methane from organic compounds.

power 3.65 million to 5.47 million homes based on the average US electricity consumption (www.eia.gov).

1.2 Methane-producing biological reactions can be unstable when confined within engineered bioreactors, and this instability is a main reason they are not used to a greater extent for the treatment of high-strength industrial organic waste streams.

Domestic sewage is a relatively low strength wastewater, with BOD values on the order of 0.2 g/L.[2] Industrial wastewater can have significantly higher BOD values (Table 1.1) and the use of MPABRs is particularly attractive for these waste streams as it produces an energy-rich biogas, does not require any external supply of oxygen (as with aerobic treatment) and produces only a small amount of sludge. In spite of these advantages, industry is often hesitant to use MPABRs due to their susceptibility to operational failure, which has been observed and documented by many researchers.[3-22] The major reason cited for the failure of anaerobic biological processes is inhibition of the methanogenic archaea due to (a) the buildup of volatile acids causing pH reduction and (b) toxic overloading of the reactor.

Table 1.1 Reported BOD concentrations in industrial organic waste streams

Industry	**BOD (g/L)**	**Reference**
Paper and Pulp Mill	0.4 – 2	[23, 24]
Textiles	0.7 – 2	[24]
Dairy	0.4 – 2.5	[24, 25]
Pharmaceutical	2 – 3.3	[23, 24]
Meat processing	0.6 – 8	[24, 26]
Sugar Manufacturing	1.7 – 10	[24]
Vegetable Oil Processing	20 – 35	[23]
Distillery	1 – 60	[24, 25, 27]

1.2.1 Volatile Acid Buildup

While there are many microbial processes involved in anaerobic digestion,[20-22, 28] the pH stability issues mainly involve the final two steps. Here, facultative heterotrophs produce volatile acids (mainly acetic acid), which are then converted to methane and carbon dioxide by anaerobic methanogens (Figure 1.1). The acetogens have a higher growth rate than the methanogenic archaea and a wider pH range for growth. When these processes are operating at steady-state, the rate of acetic acid production is balanced by the uptake rate of the methanogens and the reactor pH is maintained near neutral. An imbalance can occur when there is a sudden increase in the organic loading in the reactor through changes in either concentration or flowrate. When the organic loading increases, the acetic acid production rate increases. If the methanogens cannot keep up with the acetic acid production, it begins to build up and the reactor pH decreases. If the pH drops out of the operational range of the methanogens, their acetic acid utilization rate decreases, further exacerbating this imbalance. If the acetic acid continues to build up and the pH continues to drop, methanogenesis and methane production will cease. This is then followed by a decline in acetogenesis as the pH continues to drop, and the reactor has now failed. This process is termed “going sour” and its prevention requires active operator monitoring of the pH and alkalinity within the reactor and control via addition of sodium bicarbonate or lime.[2,20-22,25]

While the organic loading in sludge digestion at wastewater treatment plants is relatively stable, the organic concentrations and flow rates in industrial waste streams can be highly variable. Examples for two waste streams are presented in Table 1.2. Additionally, many operations are batch-based, resulting in step-function inputs into the MPABR. The stringent monitoring and control required for stable reactor operation under these conditions makes anaerobic digestion less attractive to industry compared to the options of either paying the sewer authority for inputting high-BOD wastewater or pre-treating the wastes using the more stable (and energy negative) aerobic biological treatment.

1.2.2 The built up of heavy metals

Another concern for industrial application of anaerobic digestion is the presence of toxic metal inhibitors to methanogenic archaea, including copper, nickel, zinc and chromium.[2, 6, 18-22] While pretreatment would be implemented for known inputs of toxic metals, the concern is accidental releases. And there is a particular concern for upsetting anaerobic digesters.[29] Heavy metals are quite different from other toxic substances, they are not biodegradable and can accumulate to the toxic concentrations.[30] A study of the performance of anaerobic digesters found that heavy metal toxicity can be the major causes of digester upset or failure[31].

Table 1.2 Variation in flow and BOD for two example waste streams

Waste (production unit)	Flow (gal/production unit) % Frequency[23]			BOD (lb/production unit) % Frequency[23]		
	10	50	90	10	50	90
Brewery Wastewater (bbl Beer)	130	370	600	0.8	20	44
Tomato Waste (Case)	0.50	0.75	1.00	0.15	0.40	0.65

It is believed that the heavy metals are toxic to anaerobic bacteria because they can disrupt the function or structure of enzymes through combining with thiol and other groups on protein molecules or through replacing existing metals in enzyme functional groups.[32]

While heavy metals are known to be toxic, some heavy metals are critical components in microbial enzymes and are required for numerous anaerobic biological reactions. A study of heavy metals in ten methanogenic strains showed concentrations increasing as: Fe > Zn > P > Ni > Co = Mo > Cu.[33] Overall the effects of the toxic heavy metals are controlled by the total metal concentration, chemical forms of the metals, and other factors such as pH and redox potential, with methanogens more susceptible to heavy metal toxicity than acetogens.[34-36]

The anaerobic system can be so complex that heavy metals in the system commonly involved in more than one physico-chemical processes and this process can produce different forms of heavy metals including precipitation as sulfide, carbonate and hydroxides,[34,37] sorption to the solid surface such as biomass and inert surface,[38,39] and formation of complexes in solution with intermediates and product compounds.[40-43] Even if there are numerous heavy metal forms, only free, soluble metals are toxic to the anaerobic bacteria.[37,44,45] And studies have shown that heavy metal toxicity isa better correlated to its free ionic concentration than to its total concentration.[46-48]

In most studies, however, the various physico-chemical forms of the heavy metals were rarely distinguished due to the complex interactions between the heavy metals and anaerobic sludge and/or lack of analytical techniques for separating metal species.[36,40,45,49]

Due to various physico-chemical forms, different substrates, methanogenic archaea genres, and environmental factors, the toxic concentrations of heavy metals have been reported to range from 1 mg/L to hundreds of mg/L.[29,35-37,41,47] But commonly 50% inhibition of methanogenesis caused by the heavy metals indicated that heavy metals' toxicity in the decrease order is Cu > Zn > Ni.[35,50,51] And the relative sensitivity of acidogenesis and methanogenesis to heavy metals is Cu > Zn > Cr > Cd > Ni > Pb and Cd > Cu > Cr > Zn > Pb > Ni, respectively.[50,51]

Currently, the most important methods for mitigating heavy metal toxicity are through chelating or precipitating heavy metals by the addition of chemicals or through chemical or physical adsorption by the addition of solid surface.[45] Sulfide is the main agent for precipitating heavy metals except chromate. Anaerobic reactors recovered when sulfide was added after copper exposure and when added prior to copper exposure, sulfide significantly reduced the time required for recovery.[29,36] However, sulfide can also be toxic to anaerobic digesters at high concentrations.[52] The chelation of heavy metals by organic ligands has been well documented for several metals. For example the addition of EDTA, PDA, NTA, aspartate, and citrate can decrease the toxicity of nickel.[53] The other way of mitigating the toxicity of heavy metals is the presence of solid surface in the reactor.[54] The addition of solid surface provided positive effect for mitigating the heavy metal's toxicity and is usually proportional to the solid surface area or the amount of solids. The mechanism is believed to be chemisorption.[55,56] In addition, the affinity of sludge to heavy metals has been found as: Cu > Cd > Zn > Ni.[55] Similarly, the addition of activated carbon, kaolin, bentonite, diatomite and other waste materials such as compost and cellulose pulp waste can also mitigate the inhibition of heavy metals.[57]

1.3 The application of ion exchange fibers was proposed to stabilize anaerobic reactors to upset by preventing inhibition of methanogenic archaea

The development of a simple, passive method for stabilizing MPABRs with minimal to no operator control will help make MPABRs attractive to industry. The overall goal is to greatly increase the number of companies using MPABRs to treat their high-strength wastewater and capture energy from their waste streams. To address this need, i proposed the use of weak acid ion-exchange polymers to passively buffer pH which also has the added benefit of removing toxic metals that may be introduced into the reactor due to accidental releases. The incorporation of weak acid cation (WAC) polymers to stabilize an anaerobic biological reactor is illustrated in Figure 1.2.

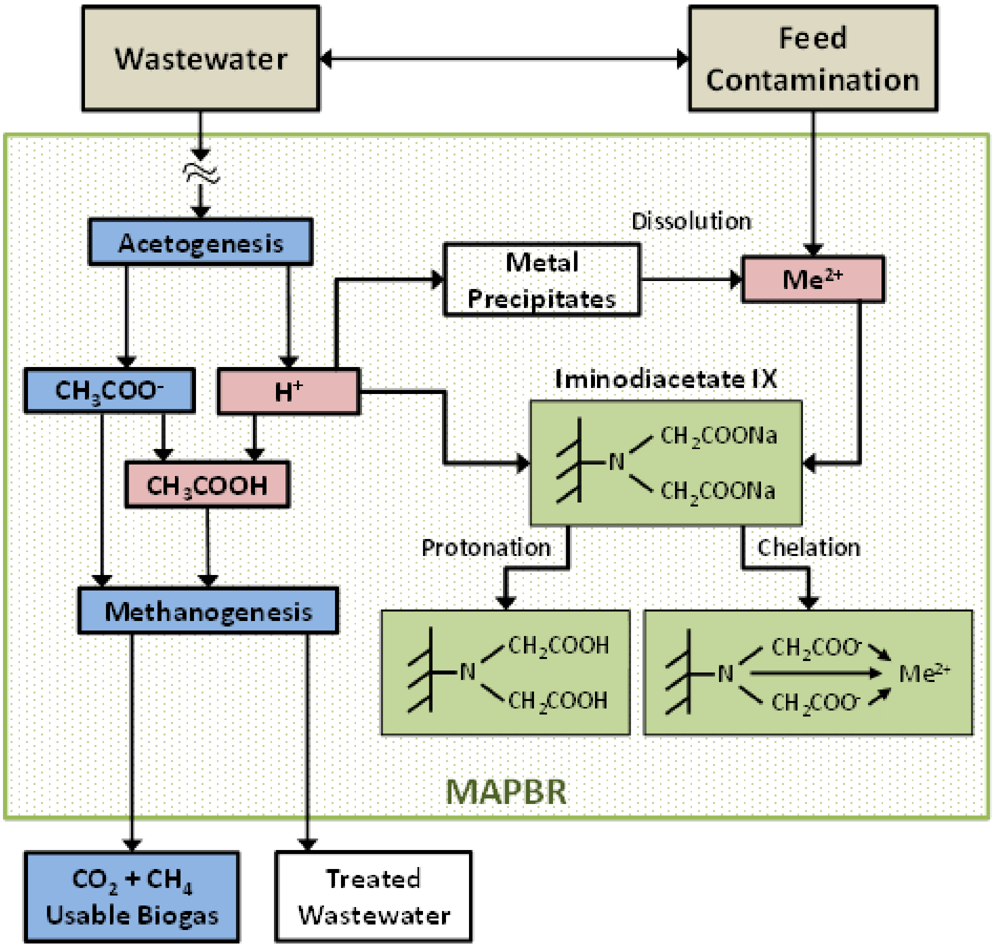

Figure 1.2 A schematic illustrating the dissipation of protons and dissolved cationic metal ions in an MAPBR stabilized by the presence of iminodiacetate ion exchange (IX) polymers.

Unlike sodium bicarbonate or lime, ion exchange polymers are insoluble and can achieve self- regeneration through a series of reactions, so it won't need continuous replenishment and monitoring. And compared to other active material, it has high regeneration rate, reaction rate, selectivity, capacity and very stable to high alkaline solution. An example of working principle of WAC with iminodiacetate functional group is described below. First the fixed sodium form WAC ion exchange fiber will react with calcium and magnesium, because WAC material has a much greater affinity for divalent ions (Equation 1.1). After excess organic matters come into anaerobic reactors, WAC ion exchange polymers will combine H^+ and release divalent ions (Equation 1.2), because they have the highest affinity to protons. Through this process, the polymers were converted to H form and avoided bulk pH to sharply decrease. Generally speaking, the use of hydrogen form WAC polymers is limited, because in its hydrogen form, the WAC ion exchange polymers can only remove cations (such as calcium, magnesium or sodium) associated with alkalinity (Equation 1.3 and 1.4). However, this limitation didn’t affect its usage in anaerobic biological wastewater treatment process.

And on the contrary, the WAC polymers just right achieved its self-regeneration with plenty of alkalinity agents after organic overloading event.

$$\mathbf{R\text{-}CH_2\text{-}N\text{-}(CH_2COONa)_2 + Ca^{2+} \rightarrow R\text{-}CH_2\text{-}N\text{-}(CH_2COO)_2Ca + 2Na^+} \qquad \textbf{Equation 1.1}$$

$$\mathbf{R\text{-}CH_2\text{-}N\text{-}(CH_2COO)_2Ca + 2H^+ \rightarrow R\text{-}CH_2\text{-}N\text{-}(CH_2COOH)_2 + Ca^{2+}} \qquad \textbf{Equation 1.2}$$

$$R\text{-}CH_2\text{-}N\text{-}(CH_2COOH)_2 + Ca(HCO_3)_2 \rightarrow R\text{-}CH_2\text{-}N\text{-}(CH_2COO)_2Ca + 2H_2CO_3 \quad \textbf{Equation 1.3}$$

$$R\text{-}CH_2\text{-}N\text{-}(CH_2COOH)_2 + CaCl_2 \leftarrow R\text{-}CH_2\text{-}N\text{-}(CH_2COO)_2Ca + 2HCl \quad \textbf{Equation 1.4}$$

Also, the example of cation-exchange reaction between dissolved nickel and calcium ions with iminodiacetate functional groups is shown below:

$$R\text{-}CH_2\text{-}N\text{-}(CH_2COO)_2Ca + Ni^{2+} \rightarrow R\text{-}CH_2\text{-}N\text{-}(CH_2COO)_2Ni + Ca^{2+} \quad \textbf{Equation 1.5}$$

Our laboratory previously examined the use of spherical ion-exchange resin beads to buffer anaerobic biological reactors.[58, 59] The conclusion was that the use of ion exchange materials may work, but that application of the technique requires an increase in the exchange kinetics, while maintaining a means to readily incorporate the materials into anaerobic biological reactors. Advances in development of ion-exchange materials have led to the production of very thin ion-exchange fibers (IXFs), on the order of 10 um diameter, with properties that make them attractive for use in anaerobic biological reactors. These thin fibers offer significantly faster sorption kinetics due to their smaller size. At the same time, IXFs allow practical installation in anaerobic reactors, where they can be easily suspended from the roof or walls of the reactor as woven sheets or as porous pillows, and as necessary, their submergence into the reactors can be varied without any major difficulty.

The IXF used in this study was the cation-exchange fiber Fiban X-1 (Institute of Physical Organic Chemistry, Belarus). Its diameter is approximately 25 um and it contains the iminodiacetate functional group, which provides pH buffering capacity across a wide pH range.[59, 60] It is shown in Figure 1.3 and its properties are summarized in Table 1.3. The iminodiacetate functional group also exhibits high affinity toward metals and commonly encountered cations in the order of $Cu^{2+} > Ni^{2+} > Zn^{2+} > Mn^{2+} > Ca^{2+} > Mg^{2+}$,[59] and thus may also be used to alleviate the impact of toxic of heavy metals, such as Ni and Cu. In addition to cationic metals, chromate is an anionic heavy metal that may be present in wastewater. To address this, I also examined the use of the strong anion ion exchange fiber Fiban A-1 (Table 1.4) to alleviate chromate toxicity to anaerobic reactors.

The addition of IXF may cause the formation of biofilm and it is believed that biofilm can help anaerobic cells resist toxic metals, compared with planktonic bacteria. One study examined the effects of the heavy metals copper, lead, and zinc on biofilm and planktonic *Pseudomonas aeruginosa* cells and it found that the biofilms were from 2 to 600 times more resistant to heavy metal stress than planktonic cells.[61] Conversely, another study examined the toxicity of 17 metal cations to biofilm and planktonic *Escherichia coli* JM109, *Staphylococcus aureus* ATCC 29213, and *Pseudomonas aeruginosa* ATCC 27853 and it showed no difference in metal toxicity between biofilms and planktonic cells.[62] To separate the effects of ion exchange and biofilm formation effect in my study, I used reactors with IXF, inert glass and polymer fibers, and no fibers

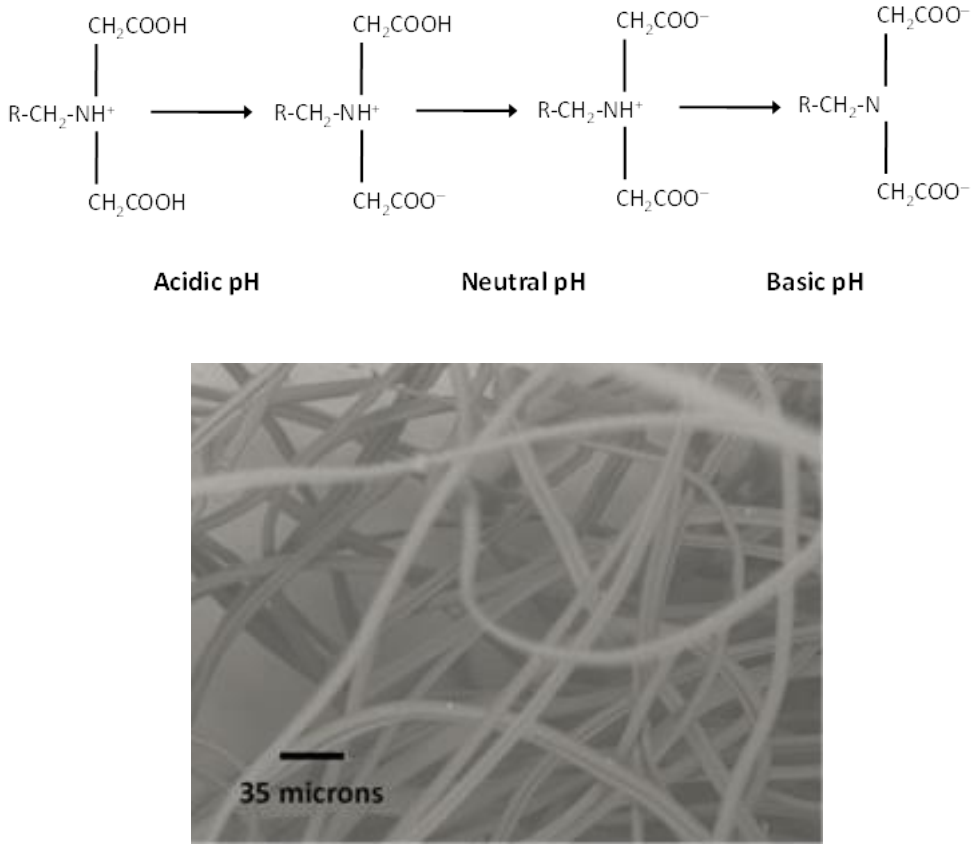

Figure 1.3 The IXF Fiban X-1 utilizes imminodiacetate functional groups attached within a polyacrylonitrile fiber. Imminodiacetate has a high buffer capacity due to its weak-acid functional groups and speciation at different pH values. It also selectively adsorbs cationic heavy metals.

Table 1.3 The properties of FIBAN X-1
(http://ifoch.bas-net.by/en/research/fiban/X1_1.html)

Functional groups	$-N-(CH_2-COO^-)_2$
Polymetric matrix	Polyacrylonitrile fiber
Physical forms	Non-woven needle-punctured fabric with surface density of 200 – 1000 g/m^2. Color: yellow.
Optimal capacity meq/g	No less 3.5 (according to -COOH).
Temperature working range	0 - 80 °C
Stability against aggressive media	Stable against concentrated HCl, H_2SO_4 and organic solvents.
Osmotic stability	Stable in cycles of acid-alkali alternative treatment and drying-wetting.

Table 1.4 The properties of FIBAN A-1
(http://ifoch.bas-net.by/en/research/fiban/A1.html)

Functional groups	$-N-(CH_3)_3$
Polymetric matrix	Polypropylene grafted with copolymer of styrene and divinyl-benzene
Physical forms	Starting textile form is staple. On special order the non-woven textile materials with surface density 250-1000 g/m^2.
Optimal capacity meq/g	No less than 2.7
Temperature working range	up to 100 °C (Cl-form) up to 50 °C (OH-form)
Stability against aggressive media	Stable at the room temperature in 0.5 N acids, alkilies, NaCl solutions. Stable in oxidizing media
pH working range	0-14

1.4 Experiment hypothesis

The hypothesis of organic overloading experiment is that IXF rapid kinetics and buffer capacity will dampen large fluctuations in pH in MPABRs by taking up protons as acid production increases and releasing them as it declines (Figure 1.4). The occurrence of a large fluctuation can increase acetic acid production beyond the ability of the methanogens to assimilate it. This causes a decrease in pH as acetic acid accumulates, resulting in a negative feedback loop that can lead to the cessation of methanogenesis and reactor failure. In contrast to active operator intervention and pH control via chemical addition, IXF can be used to passively dampen larger fluctuations in pH due to increases in organic loading, allowing the reactor to maintain operation with minimal operator oversight.

It is hypothesized that the IXF will take up a shock loading of toxic metal through selective sorption shown in Figure 1.5. The IXF will then slowly regenerate itself by releasing the metal back into solution after the shock load has dissipated, with the aqueous concentration remaining well below the toxic level during the slow regeneration period. Regeneration period length would depend on metal type and loading, reactor chemistry, and IXF characteristics.

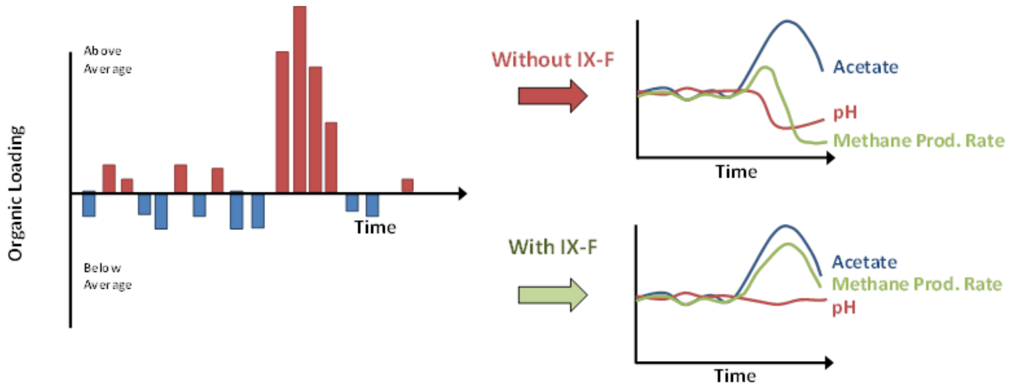

Figure 1.4 Small fluctuations in organic loading are dampened during the biological processes, with the system running at a pseudo-steady-state.

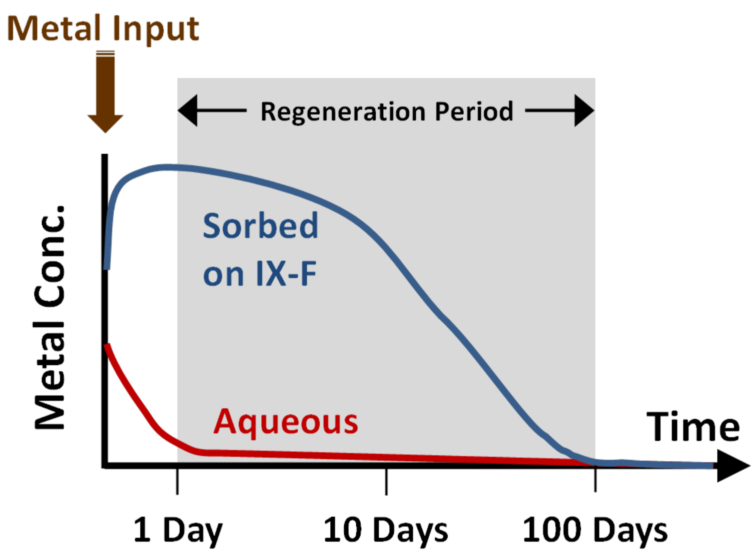

Figure 1.5 Hypothesize that the IXF will take up a shock loading of toxic metal through selective sorption.

2. MATERIALS AND METHODS

2.1 Selection of materials

Experimental objectives were to study the use of ion exchange fiber to improve the stability of anaerobic biological reactors. For simulating an anaerobic biological wastewater treatment process, lactose was chosen as experiments substrate because lactose can be easily decomposed into glucose, which is the most important intermediate in the whole anaerobic biological treatment process, especially in the fatty acids formation process. And at the same time, lactose gives us the chance to keep the most common three bacteria – the hydrolytic bacteria, acidogensis/acetogensis bacteria and methanogenic archaea together inside the anaerobic reactor in responds to two kinds of bacteria - acidogensis/acetogensis bacteria and methanogenic archaea that glucose can do. And one of the most important advantages of anaerobic wastewater treatment process is to treat high strength wastewater. Here, compared with aerobic COD concentration in the range of 250 mg/l to 1000 mg/l, this study used lactose concentration in the range of 10000 mg/l to 20000 mg/l for feeding the anaerobic reactors.

Nickel, Copper and Chromate were added into reactor to test the ability of ion exchange fiber to relieve heavy metals' toxicity. All of them were reported to be the major reason of digester fail and their toxicity to anaerobic reactors has been studied in many papers. Copper is commonly considered to be the most toxic metal to anaerobic reactor. Nickel has the highest solubility near neutral pH range among all common heavy metals, which gives ion exchange fibers chance to perform its ion exchange reaction and shows significant difference of the effluent nickel concentration between reactor with and without ion exchange fiber. Chromate is one of most frequent found anion heavy metals in the wastewater. So these three ions are representative of high / low solubility and cation/anion of heavy metals in anaerobic wastewater.

2.2 Reagents

Chemicals: Chemicals are presented in Table 2.1 and they were analytical grade, bought from Fisher and Sigma chemical company. Type 2 deionized water is used throughout this study.

2.3 Analytical methods

The experimental apparatus used in this study are shown in Figures 2.1 and 2.2. The methane volume was measured by water displacement with an NaOH CO_2 scrubber used to remove CO_2 from the gas stream (Figure 2.1 and 2.2). Measurement of COD was followed by USEPA reactor digestion method 8000. The dissolved metals were analyzed using a Perkin-Elmer 2380 atomic adsorption spectrophotometer in the flame mode for parts million range. Alkalinity in the effluent was determined by the standard procedure as outlined in the Standard Methods 2320 (APHA-AWWA-WEF, 1999).

Table 2.1 The formula of anaerobic media

Chemicals	**Concentration**[60]
$MgCl_2 \cdot 6H_2O$	300 mg/l
NH_4Cl	800 mg/l
$CaCl_2 \cdot 2H_2O$	100 mg/l
$KHCO_3$	400 mg/l
Vitamin B_{12}	0.5 mg/l
Cysteine	10 mg/l
Yeast extract	20 mg/l
$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$	42 mg/l
$Ni(CH_3CO_2)_2$	2 mg/L
NH_4VO_3	0.5 mg/l
Na_2SeO_3	0.5 mg/l
$CoCl_2$	2 mg/l
H_3BO_3	0.01 mg/l

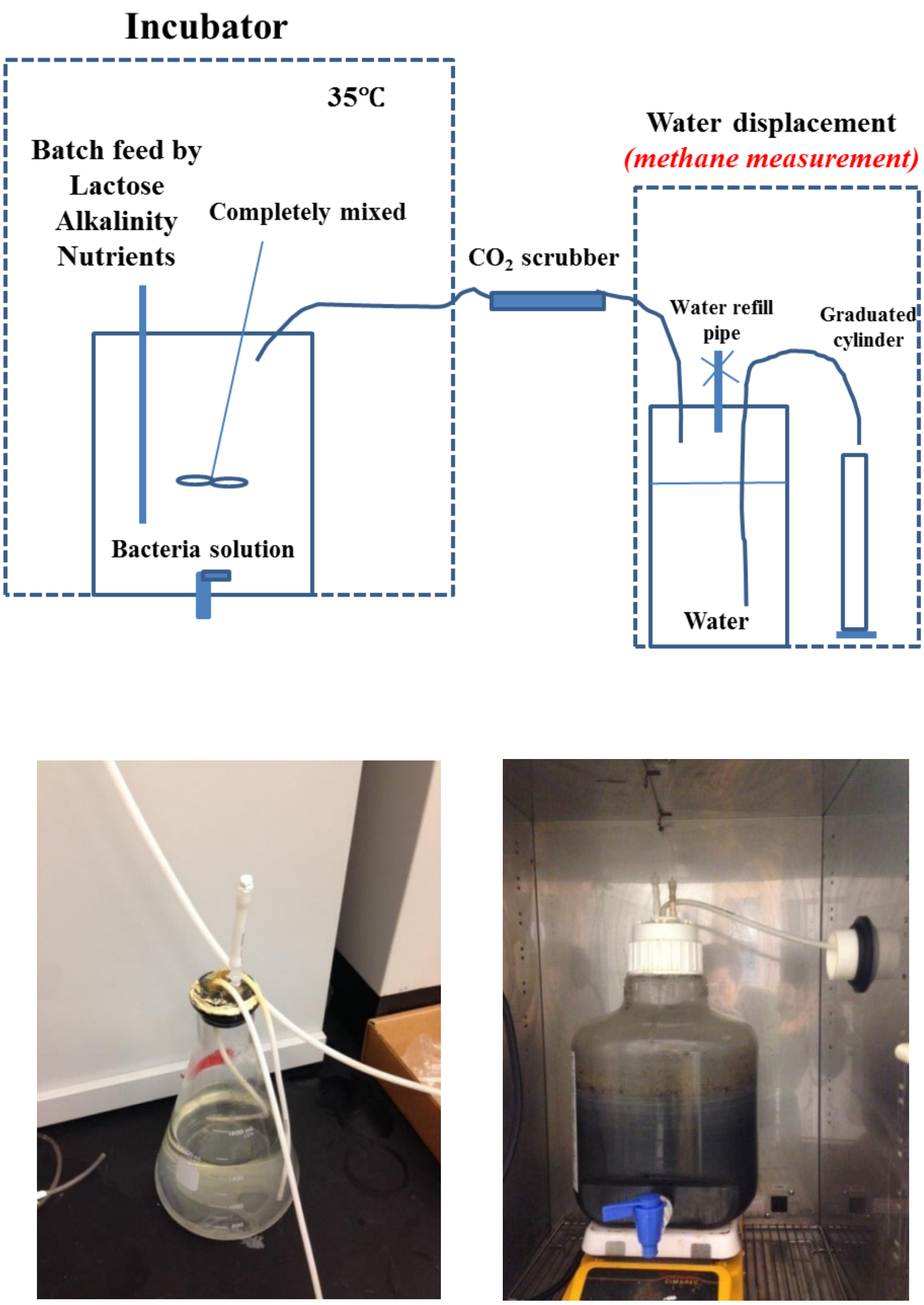

Figure 2.1 The sketch map and pictures of anaerobic mother reactor and its water displacement instrument.

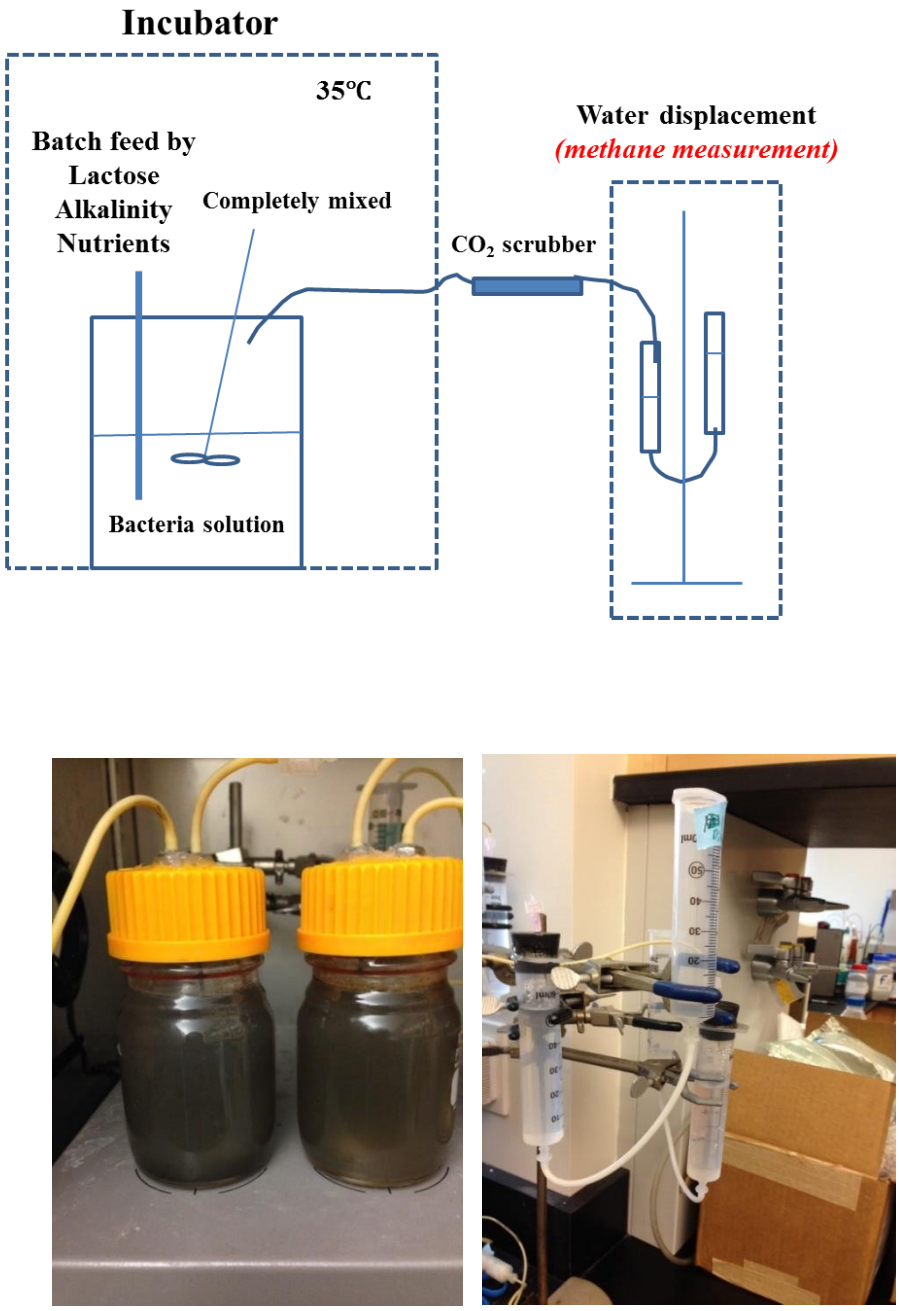

Figure 2.2 The sketch map and pictures of anaerobic test reactors and its water displacement instrument.

Mixed liquor suspended solids (MLSS) was determined from the reactor effluent as dry mass, via filtering a known volume of reactor liquid through a 0.2 μm membrane filter, followed by drying of the filtered solids at 105 ℃ and measuring the mass of solids.

2.4 Experiment objectives

In this study, there were three objectives:

2.4.1 Characterize IXF ability to stabilize anaerobic biological reactors to pH fluctuations during organic overload events

For this objective, test reactors were set up with different masses of IXF and methane generation, pH, alkalinity and COD were monitored over time. The test reactors reached their steady state when these parameters became stable. After reaching steady state, different organic overloading experiments were performed and the parameters were monitored during organic overloading event and recovery process.

2.4.2 Characterize IXF ability to stabilize anaerobic biological reactors to presence of metal loading events

Test reactors were set up with different masses of IXF and heavy metal including nickel, copper and chromate were added to the anaerobic reactors. Methane production, pH, and metal concentration were monitored over time.

2.4.3 Compare IXF and the common fiber ability to stabilize anaerobic biological reactors to organic overloading and metal overloading events

The effects of biofilm formation were examined by performing organic overloading and heavy metal experiments using neutral fibers and comparing the results to similar experiments with IXF.

2.5 Experiment method

2.5.1 The removal of oxygen

In this study, almost all of experiment needs to be done in the anaerobic biological environment, except the study of feature of ion exchange fibers, so the removal of oxygen is important to keep the anaerobic reactor work well. For this condition, all of anaerobic reactors were flushed by 99.98% purity, research grade nitrogen before running anaerobic biological experiments.

2.5.2 Start-up of mother reactor

Due to the low growth rate of methanogenic archaea, the experiments typically run for a very long time. For example, the solid retention time (SRT) of a CFSTR anaerobic reactor is commonly from 15 day to 40 day, therefore, a complete anaerobic experiment usually requires more than two to six months. To maintain an active bacterial culture, I prepared a large volume mother reactor to store and culture anaerobic bacteria at steady state. It makes me distribute the bacteria solution in the mother reactor to test reactors conveniently. Through using the mother reactor, each biological experiment running time can be saved at least 1 month, because the time for running bacteria into steady states has been significantly shorten, compare with using the bacteria from refrigerator.

A seed bacterial culture was obtained from the anaerobic digester at the Bethlehem, PA, wastewater treatment plant and maintained in a completely-mixed eight-liter reactor (termed the mother reactor). The mother reactor was operated with a 35 day solids retention time (SRT) at a temperature of 35 ℃ and it was used to provide stock bacterial cultures for use in the organic overloading and heavy metals overloading experiments. Alkalinity was added as $NaHCO_3$ and its concentration varies with the need of experiments for test reactors and lactose was provided as the carbon and energy source at a concentration of 10 g/L, step-fed once daily. The mother reactor was monitored for pH, alkalinity, methane generation and effluent MLSS to ensure stable operation. The sketch map of water displacement and mother reactor was shown in Figure 2.1. The generated gas came into the water displacement instrument through gas pipe because of increased gas pressure and CO_2 and water vapor were absorbed by CO_2 scrubber in this process. The increased internal pressure pushed the water to come out of the water bottle through water pipe. Also the schedule of start-up of mother reactor was provided in Table 2.2.

Table 2.2 The schedule of start-up of mother reactor

*Media volume	Lactose concentration	Duration time	Solution adding volume	Solution withdrawal volume
1L	0 g-lactose/L	0	0	0
2L	10 g- lactose /L	10 days	100 ml/day	0
4L	10 g- lactose /L	20 days	150 ml/day	50 ml/day
8L	10 g- lactose /L	40 days	230 ml/day	130 ml/day
8L	10 g- lactose /L	At least 2 months	230 ml/day	230 ml/day

*The media volume at the end of duration time.

2.5.3 The setup of test reactors

Almost all of biological experiments were performed by test reactors. Figure 2.2 shows the sketch map and photo of test reactors. A single long pipe was used for both feed inlet and withdraw outlet. A gas pipe was connected to water displacement system. Its work principle is similar to mother reactor's water displacement system, but it is more suitable for measuring small gas volume and operated much easier and more convenient. The test reactor's water displacement system was made of two connected syringes with marked volume. The generated methane pushed water level decrease due to increased pressure, so the difference between its water original and present level is the generated methane volume.

2.5.4 Chemistry experiment method

In order to achieve experimental objectives, ion exchange fiber was characterized. Some important feathers, such as its proton adsorption capacity, its reaction kinetics and its heavy metals removal capacity need to be investigated.

2.5.4.1 Ion exchange fiber proton adsorption and desorption kinetics

Mitra has used ion exchange resin to stabilize the anaerobic biological process. From his result, The conclusion is that the ion exchange material can help to stabilize reactor, however, its adsorption kinetics is limited by its overall adsorption rate limited by mass transfer due to large particle diameter (intra-particle diffusion). Since the organic overload event caused the accumulated volatile fatty acids and consequently the decreased pH can inhibit reactor’s methane generation, so it will be necessary to check its proton adsorption rate first.

100 ml pH=6 DI water with 1 g sodium form Fiban x-1 was mixed with 1ml 1 M HCl. pH and time were recorded to see how long the pH can reach equilibrium in the proton adsorption. After reached equilibrium, 1 ml 1 M NaOH was added into solution. Also pH and time were recorded to see how long the pH can reach equilibrium in the proton desorption process.

2.5.4.2 Fiban X-1 Nickel adsorption kinetics in anaerobic media

One of objectives indicates that the IXF can protect anaerobic reactors from heavy metals. So I need to know how fast the ion exchange fiber can absorb heavy metals. Nickel has very high solubility in the neutral pH, which provides a good condition for ion exchange fiber work. So nickel was chosen as the research target. 5 mg, 10 mg, 30 mg and 60 mg Ni from 20.43 mg, 40.86 mg, 122.586 mg, 245.17 mg $NiCl_2.6H_20$ were added into four different 100 ml anaerobic media containing 0.2 g Fiban X-1 respectively. Their equilibrium time and nickel concentrations were recorded.

2.5.4.3 Fiban X-1 Nickel removal efficiency in anaerobic media

The information of total capacity of ion exchange fiber can be searched from websites, however, in practice the fiber has not it total ability to treat water. In some high alkalinity conditions, its performance can be only 10% of its total ion exchange capacity. In addition, both the nickel adsorbed in the fiber and left in the media was detected, because only the nickel left in the aqueous phase can inhibited the methanogenic archaea.

This experiment and the nickel adsorption kinetic experiment condition were the same. But this experiment used the initial nickel concentration and aqueous nickel concentration to make figure.

2.5.4.4 Examine Fiban X-1's proton ion exchange capacity through the analysis of the cation ions at pH 6 and 7 in anaerobic media

Before the long term biological experiment, the proton ion exchange capacity of Fiban X-1 was tested. In the ion exchange process, ionic equivalents attached to the functional group of fiber should be equal to ionic equivalents detached to the fiber, so Fiban X-1 proton ion exchange capacity will be equal to the ionic equivalents which are replaced by the addition of acids. In this experiment, main cation ions are sodium, calcium and magnesium, so their ionic equivalents change with the addition of acids will be the Fiban X-1 proton ion exchange capacity. Unlike other ion exchange process, the proton ion exchange process cannot happen without pH change, so this experiment focused on the fiber's proton ion exchange capacity in a pH range instead of a pH point. Due to the feature of methanogenic archaea, the fiber proton ion exchange capacity in the pH range of 6 and 7 was espically important for anaerobic reactors.[20-22, 28] The cation ions concentration were detected at pH 7 and 6, and then the amounts of adsorbed protons can be calculated.

The mass of 0.2 g sodium form Fiban X-1 was mixed with 100 ml anaerobic media. After equilibrium, it was titrated by 1 N HCl and its pH dropped from 7 to 6. The Ca and Mg concentration were measured by atomic adsorption spectrophotometer before and after titration. The energy dispersive X-ray (EDX) technology was used to detect whether sodium was inside this fiber or not.

2.5.4.5 Examine Fiban X-1's proton ion exchange capacity through the titration of bacteria suspension

Fiban X-1's proton ion exchange capacity was also checked through titration in bacteria suspension.

Two 100 ml pH=6.95 anaerobic bacteria solutions with or without 0.18 g Fiban X-1 were titrated by 1 N HCl. The titration end point was around 5.9, which was the lowest pH for most of methanogenic archaea. The equilibrium pH and volume of HCl were recorded. Proton ion exchange capacity can be calculated through the comparison of two titration curves.

2.5.5 Long term biological experiments

2.5.5.1 Lab-scale experiments set-up

The lab-scale experiments used 125 ml bottles modified with gas and liquid sampling ports added to the cap. The reactors were seeded with bacteria from the mother reactor and then step-fed once daily with anaerobic growth media, with the lactose and alkalinity loading rates depending on the experiment. The reactors were mixed on a magnetic stirrer in an incubator at 35 ℃. The reactor effluent was analyzed for pH, alkalinity, COD, aqueous metal and the headspace gas was analyzed for methane production.

Two different types of experiments were conducted, examining the effects of (1) organic overloading and (2) heavy metals addition. For each experiment, reactors were set up with different IXF mass loadings and two were set-up without any fiber present, one of which was the control reactor and was not stressed. Additionally, a few experiments were conducted with inert fibers. The IXF used in this study was the cation-exchange fiber Fiban X-1 and anion-exchange Fiban A-1 (Institute of Physical Organic Chemistry, Belarus). Prior to each experiment, the IXF was washed in 1 N HCl, followed by adjustment of the pH to 6.9 via addition of 1 N NaOH. The IXF was formed into a ball shape and suspended in the reactor (Figure 2.3). The reactors were allowed to reach and maintain steady-state conditions, after which the overload experiment was initiated. Since the experiments are performed under different conditions, their general experiment conditions are listed in Table 2.3 and 2.4. But the specific experiment conditions were described below.

Figure 2.3 The configuration of ion exchange fiber hung in the tube.

Table 2.3 Summary of organic overloading experiment conditions

Test #	HRT	Alkalinity	Influent lactose concentration	Period	With common fiber
Test 1	Not change	Decreased to 0	4 times	1	No
Test 2	Not change	Not change	0~48 times	1	Yes
Test 3	1/3 times	Not change	Not change	1	No
Test 4	Not change	Decreased to 0	3 times	2	Yes
Test 5	Not change	Decreased to 0	3 times	1	Yes

Table 2.4 Summary of heavy metals overloading experiment conditions

Test #	Metal Name	Period	With common fiber
Test 1	Nickel	1	No
Test 2	Nickel	1	Yes
Test 3	Copper	1	Yes
Test 4	Chromate	1	No
Test 5	Copper	2	No

2.5.5.2 Organic overload experiment #1

To assess the performance of Fiban X-1, the first organic overload event simulated excessive organic loading into anaerobic reactors without supporting of alkalinity. Mass loadings of 2 g/L and 10 g/L Fiban X-1 were added into two different reactors. For comparison, two reactors were used without any IXF present, one of which was the control reactor and was not stressed.

In the organic overloading experiment, the reactors were provided lactose at a baseline loading of 168 mg/L-d (influent concentration of 4.5 g/L) and alkalinity at a baseline $NaHCO_3$ loading of 93.6 mg/L-d (influent concentration of 2.5 g/L). The reactors contained 80 mL of liquid and were step-fed once daily with 3 mL of growth media, providing a solids retention time (SRT) of 26.7 days. After reaching steady-state conditions (approximately 30 days) the lactose loading was increased from the steady-state value of 168 mg/L-d to an overload of 675 mg/L-d, with the alkalinity loading simultaneously reduced from the steady-state value of 2.5 g/L-d of $NaHCO_3$ to zero from day 36 to 46. This organic overloading was continued until the reactor without IXF present failed (methane generation ceased), at which time the influent lactose and alkalinity were returned to the baseline loadings and the system was monitored for its ability to recover.

2.5.5.3 Organic overload experiment #2

This experiment was set up to determine the influence of the addition of solid fiber. The first experiment did not consider that the fiber itself can affect the stability of anaerobic reactors during organic overloading. It was observed that bacteria aggregated at the IXF surface during the first organic overloading experiment, so the accumulated bacteria also may enhance reactor's organic consumption ability.[64] Then in this experiment design, similar to the first experiment, two reactors without any IXF present, one of which was the control reactor and was not stressed. IXF was loaded at 1 g/L and 6 g/L two different reactors and for comparison, 15 g/l glass fiber, which is mainly made of inorganic elements, is also added into a reactor.

Bacteria solution volume was 100 mL and its hydraulic retention time is 33.3 days. Organic loading rate was 300 mg-lactose/L/day with the 10 g-lactose/L influent lactose concentration. During the organic overload events, organic loading rate became variable on the reactors from days 60 to 66, which their loading rate were ranged from 0-14.4 g/L/day, based on reactor volume. Reactors were step-fed once daily and 4 g/L $NaHCO_3$ was added into each reactor to provide reactor buffer capacity. The schedule of lactose concentration in this overload experiment is shown Figure 2.4.

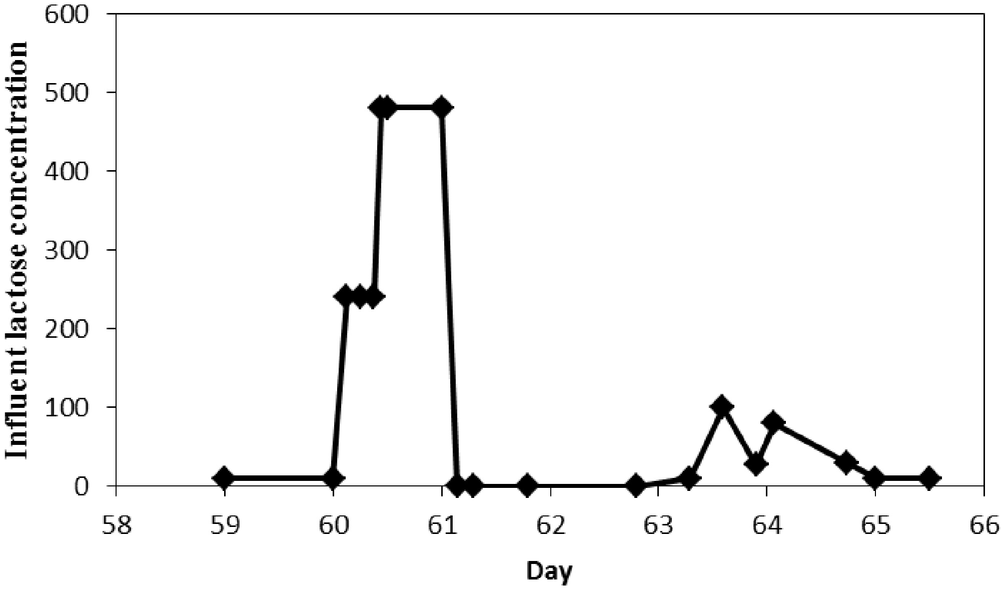

Figure 2.4 The schedule of lactose concentration change in the second organic overload experiment

2.5.5.4 Organic overload experiment #3

In this experiment, another different type of organic overload was conducted. Rather than increasing the lactose concentration, the lactose loading rate was increased by decreasing the hydraulic retention time (HRT). Similar to the previous experiments, four anaerobic reactors were run in this experiment. Two reactors without any IXF present, one of which was the control reactor and was not stressed, and two reactors with 2 g/L and 5 g/L. Bacteria solution volume was 100 mL and its hydraulic retention time was 15 day. The organic loading rate was 666.7 mg-lactose/L/d, based on reactor volume. Influent lactose concentration was 10 g/L. Organic overloading was applied into reactors during days 21.5 to 27, which decreased the HRT to 5 days. Reactors were step-fed every 12 hours and 4 g/L $NaHCO_3$ was added into reactor to provide reactor buffer capacity.

2.5.5.5 Organic overload experiment #4

The result of the second organic overload experiment demonstrated that the presence of glass fiber provided some stability to organic overloading. To explore this further, polyacrylonitrile (PAN) fiber with similar characters with Fiban X-1, but without any ion exchange functional groups, was placed into reactors.

In addition, this organic overload experiment as also designed to demonstrate the ion exchange fiber remains active over repeat organic loadings. Two organic overloading events were applied into an organic overload experiment and allowed anaerobic reactors to have an over three-week interval between two organic overloading events, which provided plenty of time for fiber's regeneration.

In this organic overload experiment, there are four kinds of reactors, which are reactors without fibers, with 5 g/L glass fiber, with 5 g/L PAN fiber and with 5g/L FIBAN X-1. All reactors were step-fed daily with lactose at a loading of 532 mg/L/d (influent concentration of 15 g/L) and alkalinity at a $NaHCO_3$ loading of 160 mg/L/d (influent concentration of 4.5 g/L). The reactors contained 95 mL of liquid and were step-fed once daily with 3.3 mL of growth media, providing a SRT of 28 days. After reaching steady-state conditions (approximately 30 days), the lactose loading was increased from the steady-state value of 532 mg/L-d to an overload of 1596 mg/L/d, with the alkalinity loading simultaneously reduced from the steady-state value of 4.5 g/L/d of $NaHCO_3$ to zero. This organic overloading event lasted 8 days from day 36 to 43 and pH, methane generation and COD was monitored. The second organic overloading event was run from day 68 to 75 and lasted 8 days.

2.5.5.6 Organic overload experiment #5

In this organic overload experiment, it is designed to compare with the fourth organic overload experiment and the experiment's conditions were very similar. The only difference was that this experiment had a higher lactose influent concentration. This experiment was designed to show how the initial lactose concentration affects the performance of anaerobic reactors during organic overload event.

There were four kinds of reactors, which are reactors without fibers, with 5 g/L glassfiber, with 5 g/L PAN fiber and with 5 g/L FIBAN X-1. Each reactor was duplicated. All reactors were step-fed daily with lactose at a loading of 709 mg/L/d (influent concentration of 20 g/L) and alkalinity at a $NaHCO_3$ loading of 160 mg/L-d (influent concentration of 4.5 g/L). The reactors contained 93 mL of liquid and were step-fed once daily with 3.3 mL of growth media, providing a SRT of 28 days. After reaching steady-state conditions (approximately 30 days), the lactose loading was increased from the steady-state value of 709 mg/L/d to an overload of 2185 mg/L/d, with the alkalinity loading simultaneously reduced from the steady-state value of 4.5 g/L-d of $NaHCO_3$ to zero. This organic overloading last 5 days and pH, methane generation was monitored.

2.5.5.7 Nickel overloading experiment #1

To evaluate the performance of Fiban X-1 during the heavy metal overload experiment, all of experiments simulated that small amount of heavy metals in the wastewater accidentally dropped into anaerobic reactors. For addressing the heavy metal overload event, plant has already added Fiban X-1 into anaerobic reactors.

The high solubility of nickel provides the chance to show the effluent nickel difference between the reactors with fiber and without fiber. This is the most straightforward way to demonstrate if the ion exchange fiber's ion exchange capacity works inside the anaerobic reactor. SEM-EDX was used to detect whether nickel comes into the fiber after biological experiment.

In this nickel overloading experiment, 4 g/L Fiban X-1 was added into one reactor. And also for comparison, two reactors without any IXF present, one of which was the control reactor and was not stressed. The reactors were step-fed daily with lactose at a loading of 667 mg/L/d (influent concentration of 10 g/L) and alkalinity at a $NaHCO_3$ loading of 267 mg/L/d (influent concentration of 4 g/L). The reactors contained 100 mL of liquid and were step-fed twice daily with 3.3 mL of growth media, providing a SRT of 15 days. After reaching steady-state conditions (approximately 30 days), a shock load of nickel of 12.15 mg of $NiCl_2 \cdot 6H_2O$ (30 ppm) was added to the anaerobic reactors at days 42.5. The system was then monitored over time for its ability to recover from the shock-loading of nickel. Nickel inside this fiber was detected by EDX.

2.5.5.8 Nickel overloading experiment #2

Similar to the organic overload, inert fiber was also tested in this nickel overload experiment. Two reactors without any IXF present, one of which was the control reactor and was not stressed. IXF at loadings of 2 g/L and 8 g/L were placed in two different reactors and for comparison, 8g/L glass fiber is also added into a reactor.

In this experiment, glass fiber was added into nickel overload experiment. The reactors were step-fed daily with lactose at a loading of 333 mg/L/d (influent concentration of 10 g/L) and alkalinity at a $NaHCO_3$ loading of 133 mg/L/d (influent concentration of 4 g/L). The reactors contained 100 mL of liquid and were step-fed once daily with 3.3 mL of growth media, providing a SRT of 30 days. After reaching steady-state conditions (approximately 30 days), a shock load of nickel of 20 ppm nickel was added to the anaerobic reactors at days 31, 33, 34.

2.5.5.9 Copper overload experiment #1

In this experiment, two reactors without any IXF present, one of which was the control reactor and was not stressed. 1.5 g/L and 6 g/L Fiban X-1 were added into two different reactors. And also for comparison, 6 g/L glass fiber was added into a reactor. Bacteria solution volume was 100 mL and HRT is 30 day. Organic loading rate was 333 mg/L/d with 10 g/L influent lactose concentration. Copper at a concentration of 10ppm as Cu^{2+} was added into reactor at days 34 and 36. The reactors were step-fed once daily and 4 g/L $NaHCO_3$ was added into reactor to provide reactor buffer capacity.

2.5.5.10 Chromate overload experiment #1

Chromate is a commonly-encountered anionic heavy metal in wastewater. In this experiment, $K_2Cr_2O_7$ was used as inhibitor to test the ability of stabilizing anaerobic reactor through the use of anion ion exchange fiber-Fiban A-1 through the use of 2 g/L and 6 g/L anion ion exchange fiber-Fiban A-1. For comparison, two reactors without any IXF present, one of which was the control reactor and was not stressed. Bacteria solution volume as 100 mL and the SRT was 29.4 days. Lactose loading rate was 340 mg/L/d. Chromium was added at a concentration of 25 ppm as chromate into the reactors at day 32, 33, 34. The reactors were step-fed once daily and 4g/L $NaHCO_3$ was added into each reactor to provide reactor buffer capacity.

2.5.5.11 Copper overload experiment #2

This copper overload experiment was performed to demonstrate that the ion exchange fiber can be used with multiple copper loadings. Two copper overloading events were applied, with the reactors allowed to recover before the second overloading event was initiated. In this experiment, 2 g/L and 8 g/L Fiban X-1 were added into two different reactors. For comparison, two reactors were run without any IXF present, one of which was the control reactor and was not stressed.

Bacteria solution volume was 100 mL and the SRT was 29.4 days. Organic loading rate of lactose was 340 mg/L/d. Copper was added into reactor at days 32, 33, 93 and 94 at a concentration in the influent of 882 mg/L. The reactors were step-fed once daily and 4 g/L $NaHCO_3$ was added into each reactor to provide reactor buffer capacity.

2.6 Aging of Fiban X-1

To demonstrate the feasibility of using IXF to buffer anaerobic reactors to metal inputs, the effects of IXF aging within the anaerobic reactors on the ion exchange capacity must be assessed. To do this, 100 mL anaerobic reactors with 0.2 g of Fiban X-1 were operated for up to one year with an influent lactose loading of 10 g/L. The fibers were removed at 4, 8 and 12 months and placed into 100 mL of anaerobic growth media at a pH of 6.5. Nickel was added to each reactor at a concentration of 300 mg/L as $NiCl_2.6H_20$ and the equilibrium aqueous concentration of nickel was determined by AA spectrophotometer.

3. EXPERIMENTAL RESULTS

Like discussed before, the organic overloading and heavy metals overloading events can affect anaerobic reactor's wastewater treatment efficiency and methane generation. If these problems are not treated correctly, anaerobic reactors will be most likely to fail in a short time and recovery of the reactor can be very expensive. In addition, it is generally believed that methanogenic archaea are much more susceptible to heavy metals and pH than the acetogenic bacteria, so both organic overloading and heavy metals overloading events can drop the reactor's pH, which will affect the methanogenic archaea to a greater effect than the acetogenic bacteria. Additionally, since the methanogenic grow rate is very slow, reactor recovery after re-establishing a neutral pH will be time consuming. In this study, the ion exchange fiber was used to help anaerobic reactors against the heavy metals and organic overloading events, and the experimental results are shown below.

3.1 Characterization of Fiban X-1

3.1.1 The proton reaction kinetics of Fiban X-1

From Figure 3.1, After HCl was added into the solution containing Fiban X-1, the pH of solution dropped from 6.0 to 3.5 immediately. In the next five minutes, reactor's pH gradually increased and came to its pH steady state 4.31. In the fifteenth minutes, NaOH was

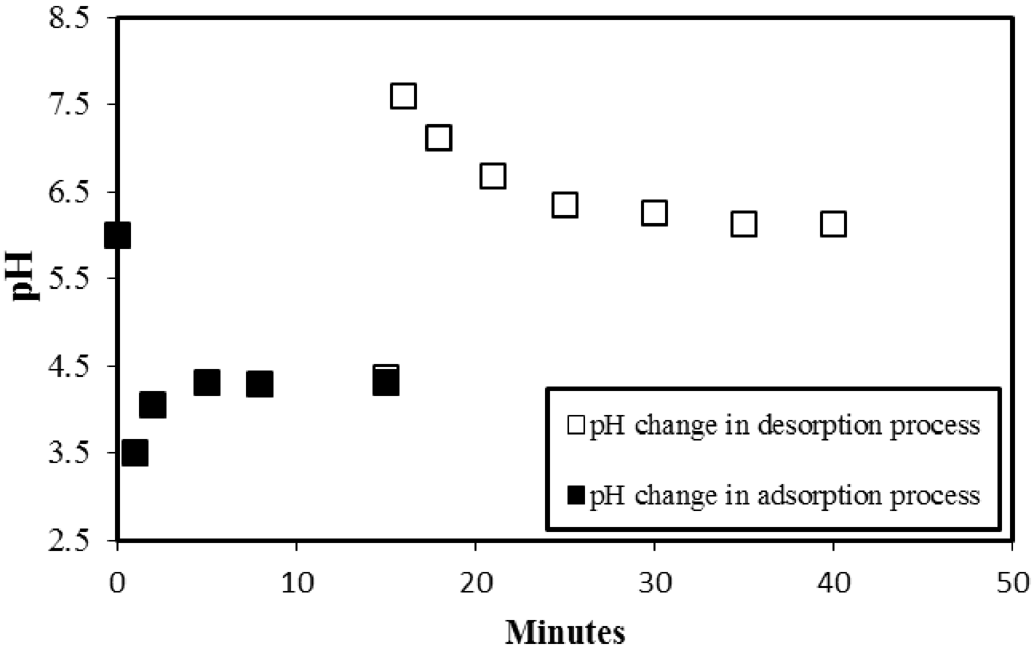

Figure 3.1 The result of Fiban X-1's proton reaction kinetics

added into solution so solution's pH increased to 7.6 immediately. Then, reactor's pH gradually decreased and came to its steady state 6.02 in fifteen minutes. Compared with often use ion exchange resin beads, Fiban X-1's proton sorption speed was much faster. For ion exchange resin beads, it need over 5 hours, even a day to finish the proton adsorption process. This result is good fit to the description from manufacture, which the fiber is commonly over 100 times faster than resin beads.

3.1.2 Fiban X-1's nickel adsorption kinetics and removal efficiency

From the Figure 3.2, the nickel adsorption rate was followed by first-order reaction and the rate constant is 1.267 $hour^{-1}$. It meant that the fiber can reduce a half of nickel concentration in 33 minutes. From Figure 3.3, the Fiban X-1 nickel removal efficiency was always higher than 90% under different conditions. When the initial aqueous nickel concentration was 100 mg/l, its nickel remove efficiency was still over 99% after equilibrium. Even when the reactor initial aqueous nickel concentration was 600 mg/L, its nickel remove efficiency was still over 90% after equilibrium. Due to the high rate constant and removal efficiency, it was deserved to try the IXF Fiban X-1 in the anaerobic biological experiment.

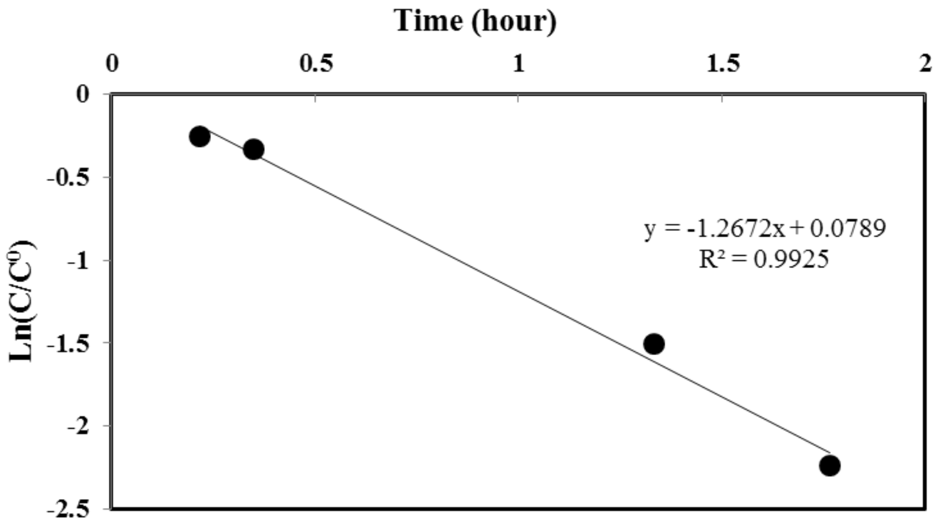

Figure 3.2 The result of Fiban X-1's nickel adsorption kinetics in anaerobic media

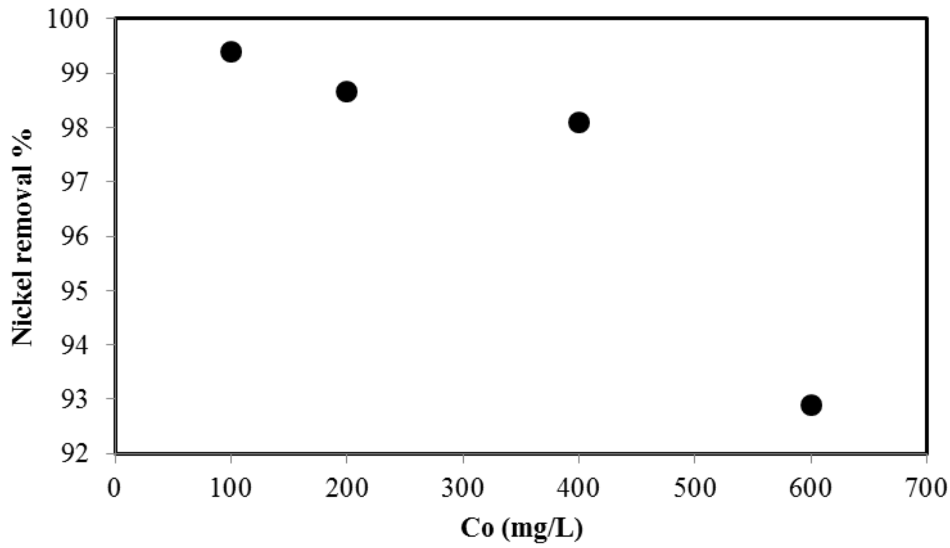

Figure 3.3 The result of 2 g/L Fiban X-1's nickel removal efficiency in anaerobic media

3.1.3 Examine Fiban X-1's proton ion exchange capacity through the analysis of the cation ions at pH 6 and 7 in anaerobic media

Unlike other ion exchange process, the proton ion exchange process cannot happen without pH change,so this experiment focused on the fiber's proton ion exchange capacity in a pH range instead of a single pH value. It is generally believed that when pH is below 6.0, methanogenic archaea will be sever inhibited.[21] So if an anaerobic reactor' pH was dropping, action must be taken to protect the digestor, at least before the pH drops below 6.0. Here, the exchange capacity of the IXF Fiban X-1 was examined as the pH dropped from 7 to 6 and the results are presented in Figure 3.4. The concentration difference between pH 6 and 7 was Fiban X-1 proton ion exchange capacity in the pH range of 6 and 7. To get its capacity, three main cation ions Na^+, Ca^{2+} and Mg^{2+} in the media were measured in different pH conditions. Ca^{2+} and Mg^{2+} concentrations were measured by AA spectrophotometer. Sodium was not found inside the fiber through the use of EDX-SEM. Thus, the cation molar equivalents were equal to the sum of Ca^{2+} and Mg^{2+}. Through calculation, Fiban X-1's proton adsorption capacity was 0.6 meq/g in the pH range of 7 to 6.

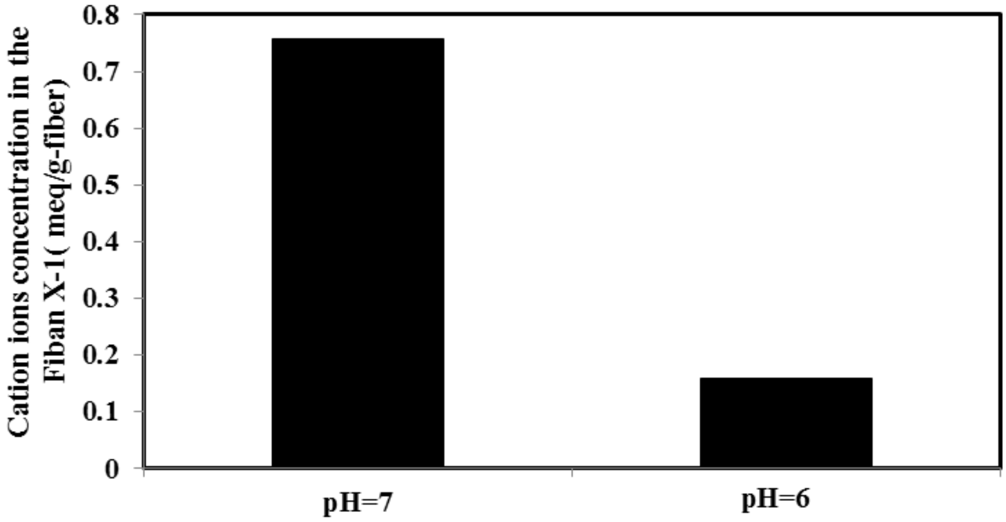

Figure 3.4 The total concentration change of Ca^{2+} and Mg^{2+} ions at pH 6 and 7 in anaerobic media

3.14 Examine Fiban X-1's proton ion exchange capacity through the titration of bacteria suspension

Fiban X-1's proton ion exchange capacity was also checked through titration in the bacteria suspension. The solid and hollow circles in Figure 3.5 represent the bacteria suspension and demonsrate that upon addition of HCl, the suspension with ion exchange fiber had a smaller pH change than the suspension without ion exchange fiber. And at the same time, their pH difference (squared data used the right axis) increased from 0 to 0.27 with the addition of HCl. Also after calculation, the Fiban X-1's proton adsorption capacity from pH 6.95 to 6.0 was roughly 0.55 meq-H/g-fiber, which was similar to the number provided in Section 3.13.

3.2 Buffering of pH due to Organic Overloading

3.2.1 The result of first organic overload experiment

Results from the first organic overloading were provided in Figure 3.6, including reactor alkalinity, pH and methane production rate. In this experiment, the performance of reactors with different mass of Fiban X-1 was compared. The organic overloading was conducted from days 36 to 46 (depicted by the gray shading) and the three stressed reactors all demonstrated a decrease in alkalinity and pH during the stress period. The methane production rate initially increased due to the increased organic loading rate, and the rate then decreased over time as the pH decreased in the reactors.

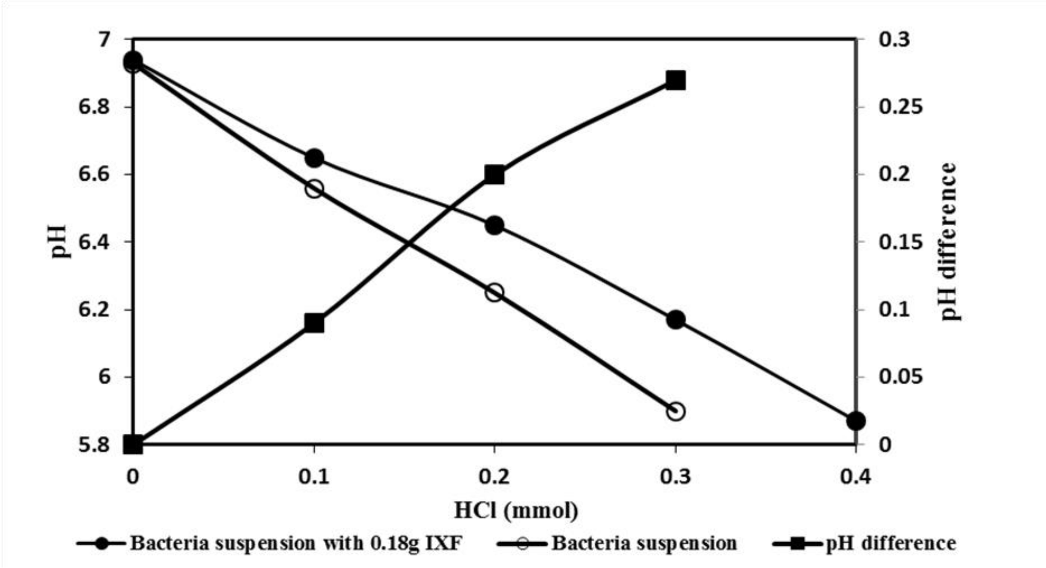

Figure 3.5 Titration of Fiban X-1 in bacteria suspension

As seen in Figure 3.6, the reactor with no IXF failed at day 46 (methane generation ceased). At this time, the pH in the reactor with no IXF had decreased to approximately 5.5, whereas the pH in the 2 g/L IXF and 10 g/L IXF reactors had decreased to approximately 6.1 and 6.6, respectively. The methane generation in the 2 g/L reactor was moderately impacted during the stress period. The methane generation in the 10 g/L reactor increased, indicating that the methanogens were not impacted by the minor pH decrease and they were able to utilize the increase in acetate production.

Upon cessation of the stress period, the two reactors containing Fiban X-1 were able to recover (i.e., pH, alkalinity and methane production returned to the pre-stress levels), whereas the reactor without fiber was not able to recover. The rise in methane production rate above the steady-state value for the 2 g/L reactor from days 56 to 67 suggested that the methanogens were able to utilize the substrate remaining in the reactor once the pH rose above approximately 6.4.

3.2.2 The result of second organic overload experiment

In the last experiment, it was observed that there were bacteria attached at the surface of IXF and a reference indicated the attached bacteria were able to help the reactor against organic overloading experiment.[63] So in this experiment, a kind of fiber without ion exchange capacity called glass fiber was added into an anaerobic reactor. Its performance was

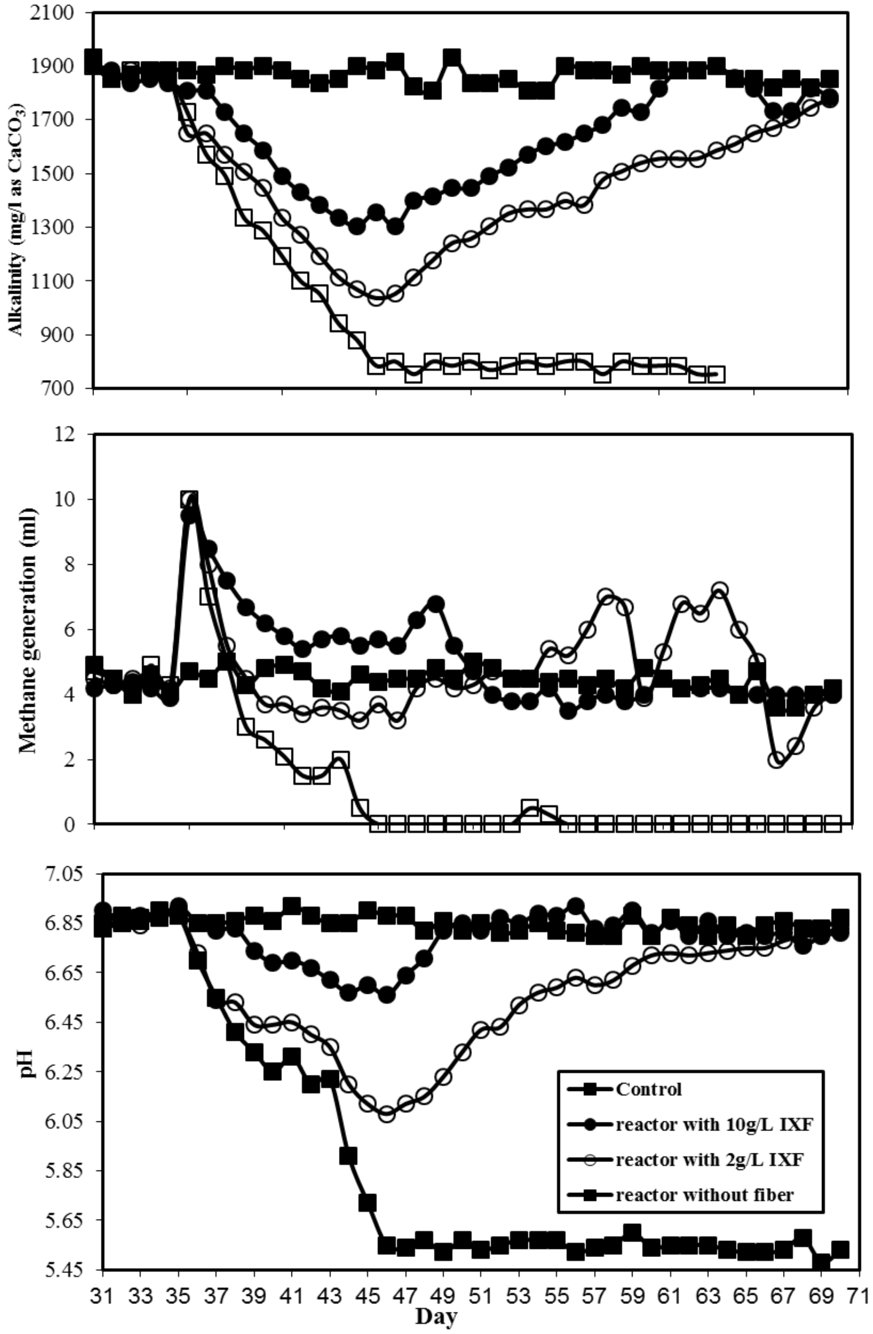

Figure 3.6 The performance of reactors with different mass IXF during lactose overloading rate

compared to reactors containing 1 g/L and 6 g/L IXF in the second organic overloading experiment. And from the Figure 3.7, the organic overload event happened in the gray region. The reactor's normal lactose feeding rate was 10 g/L/d and at day 61, the concentration was increased to 240 g/L and 480 g/L. The increase in organic loading resulted in an immediate increase in methane production (the steep slope of cumulated methane generation). But at that time the reactor's pH was also decreasing. At the end of day 61, the reactors without fiber, with 15 g/L glass fiber and with 1 g/L and 6 g/L IXF's pH were 5.31, 5.57, 5.54 and 5.9, respectively. Then the lactose concentration was decreased to zero from day 61 to 63. It should be noted that while the four data points indicated that the lactose concentration is zero from day 61 to 63, the 3.3 mL bacteria solution in these anaerobic reactors was still replaced by the fresh media with no lactose. That means that at those four data points, the $NaHCO_3$ was also added into reactors. But even if only the positive action (the addition of $NaHCO_3$) was performed from day 61 to 63, its pH didn't increase and the cumulated methane generation was zero. The pH of the other three reactors increased and small amounts of methane were also generated from day 61 to 63. From day 63 to 66, various lactose concentration ranged from 10 g/L to 100 g/L was applied into reactors. During these day, the pH of reactor with 1 g/L IXF and 15 g/L glass fiber had a downward trend and the reactor with 6 g/L IXF flucuated because the reactor with 6 g/L IXF had the better proton adsorption capacity than the other two reactors.

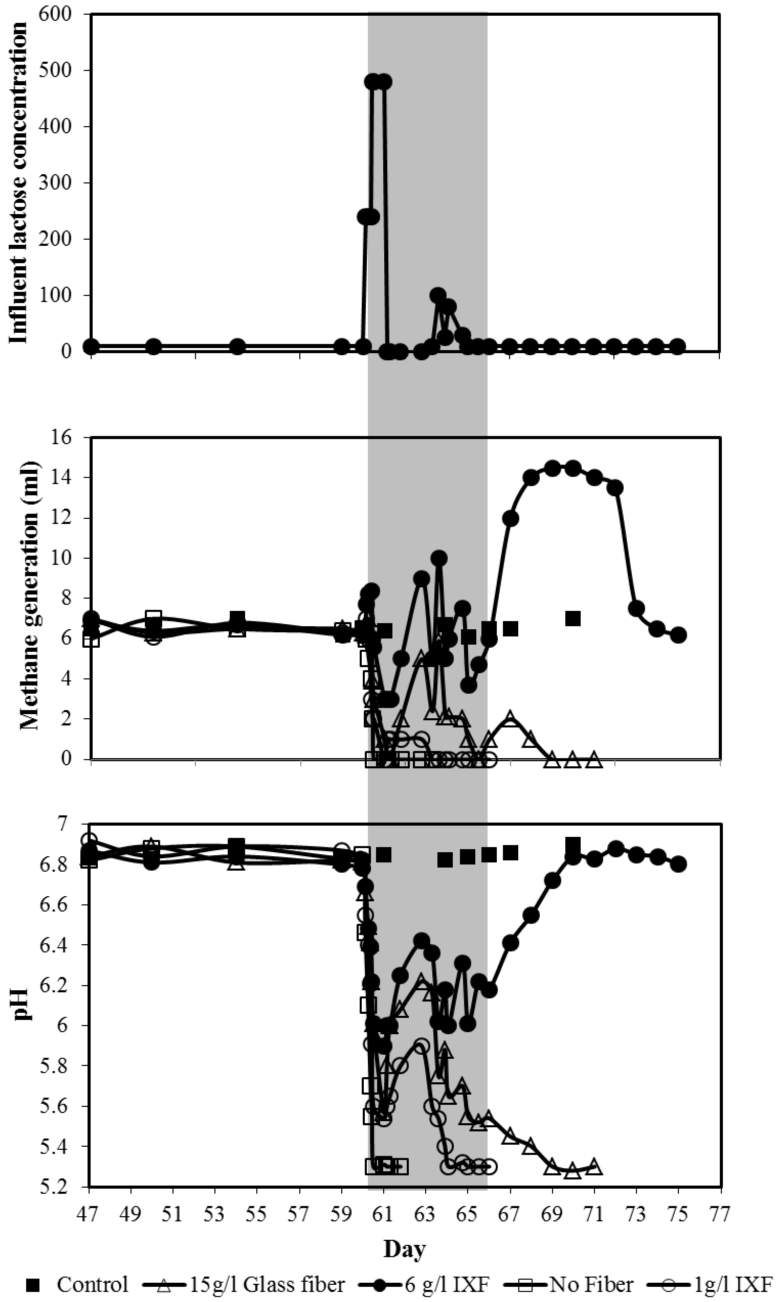

Figure 3.7 The performance of reactor with IXF and reactor with glass fiber

At the end of day 66, the pH of reactors with glass fiber, 1 g/L IXF and 6 g/L IXF were 5.54, 5.3 and 6.18, respectively. Their cumulated methane generation were 30.5 mL, 57.2 mL and 113.1 mL, respectively. After the day 66, even the organic overloading stress was removed, the pH of reactors with 1 g/L IXF and 15 g/L glassfiber were still decreasing and their cumulated methane generation didn't increase. But because that 6 g/L Fiban X-1 can provide enough proton adsorption capacity to relieve the stress of organic overloading event, the reactor with 6 g/L Fiban X-1 survived from the organic overloading event. Then its pH and cumulated methane generation showed a significant increase, because of the consumption of accumulated fatty acids. And the data showed eventually this reactor came to its steady state at day 74. As shown above, the reactor with 15 g/L glass fiber had a better performance than the reactor with 1 g/L IXF, which meant the attached bacteria were able to help reactors against organic overloading event. But the reactor with 6 g/L IXF still had a much better performance than the reactor with 15 g/L glass fiber, even if it had much smaller surface area and mass than glass fiber.

3.2.3 The result of third organic overload experiment

The third organic overloading experiment was to show the performance of reactors meeting suddenly decreased HRT was shown in Figure 3.8, including data of COD, methane generation rate and pH. The first and second organic overloading experiments had

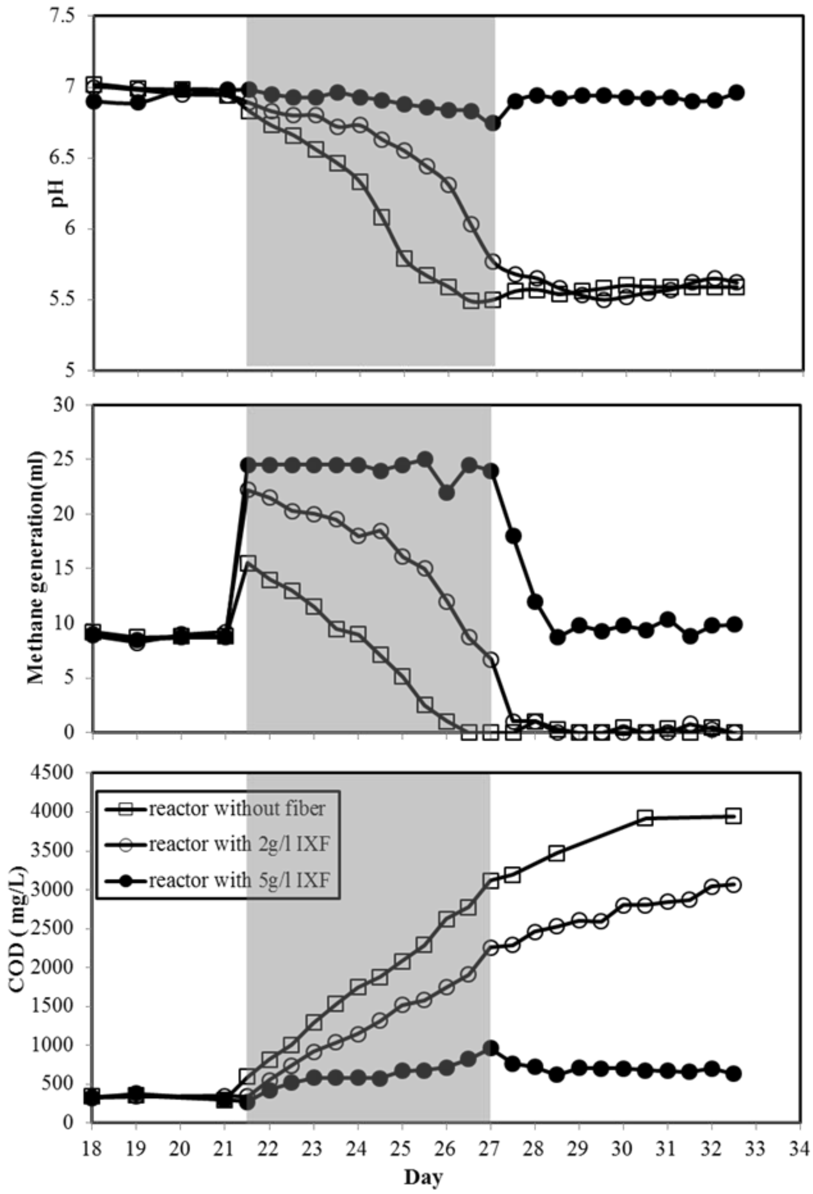

Figure 3.8 The performance of reactors meeting suddenly decreased HRT.

demonstrated that IXF can help reactors against the sudden increased organic concentration, so in third experiment, the organic overloading evet was created by decreasing the HRT of reactors, instead of increasing influent lactose concentration. The result was quite similar to the first and second experiments. The organic overloading was conducted from days 21.5 to 27 (depicted by the gray shading). Compared with the reactor containing no fiber, the two reactors with fiber obviously had a better performance including higher methane generation, lower pH and COD. Furthermore, compared with the reactor with 2 g/L IXF, the reactor with 5 g/L IXF has much better performance. So, through the results of experiment 1, 2 and 3, the more the addition of IXF, the higher the chance of reactor can survive during such an organic overloading event.

3.2.4 The result of fourth organic overload experiment

The fourth organic overloading experiment was used to show Fiban X-1 can be used in a long term, so two same events were applied into this experiment and they were conducted from days 36 to 43 and from 68 to 75 (depicted by the gray shading). Result were provided in Figure 3.9, including data of COD, pH and methane production rate. The four stressed reactors all demonstrated a decrease in pH during the first event period. The methane production rate initially increased due to the increased organic loading rate, and it then decreased as the pH decreased in the reactors. At the same time all of reactors' COD

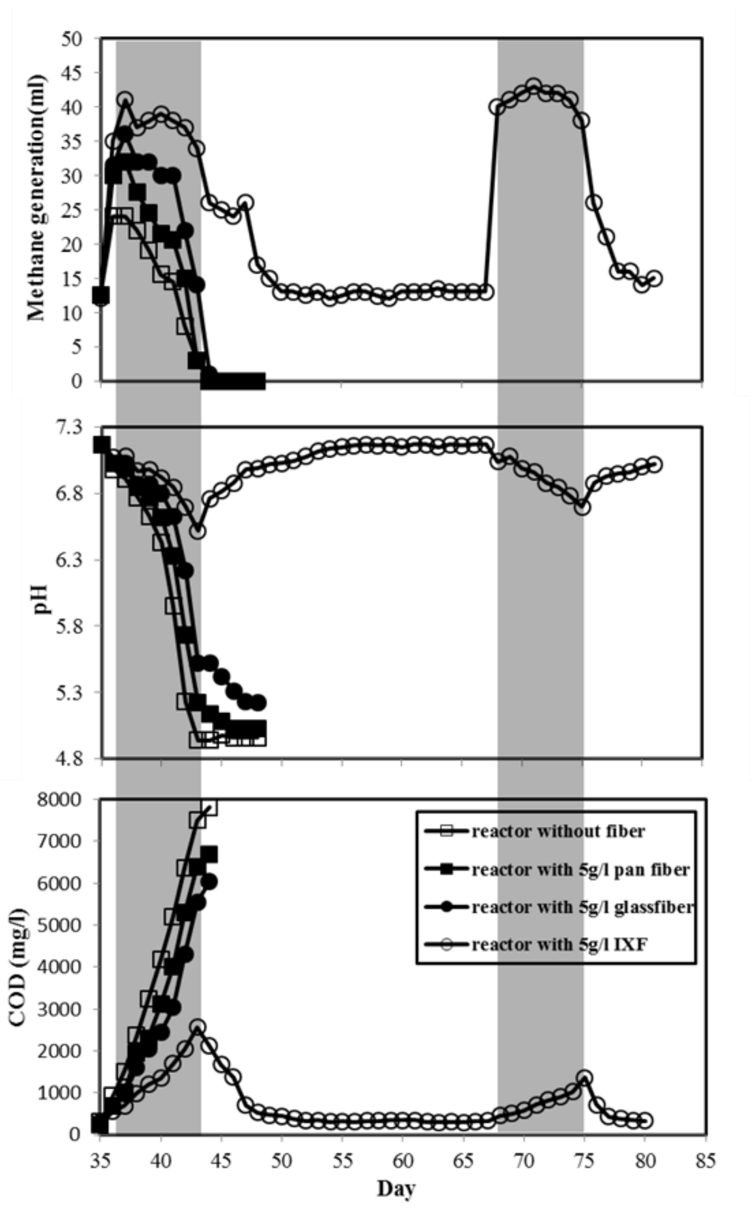

Figure 3.9 The performance of reactors response to long term organic overload event

concentration increased because the increased influent COD concentration has exceeded anaerobic reactors wastewater treatment capacity.

Except the reactor with 5 g/L IXF, all of the reactors failed at day 44 (methane generation ceased). At this time, the pH in the reactors containing no fiber, PAN fiber, and glass fiber decreased to approximately 4.94, 5.14 and 5.52, respectively, whereas the pH in 5 g/L IXF reactors decreased to approximately 6.76. The methane generation in the 5 g/L IXF reactor increased, indicating that the methanogens were not impacted by the minor pH decrease.

After stress was removed, the reactor containing Fiban X-1 were able to recover (i.e., pH, COD and methane production returned to the pre-stress levels), whereas other reactors were not able to recover. In addition, the reactors with 5 g/L PAN fiber and 5 g/L glass fiber showed a better performance than the reactor containing no fiber, including higher methane generation, higher pH and lower COD. As discussed earlier, that was likely due to bacteria attached on the solid surface, which were able to help the reactor overcome the organic overloading event.[64]

The second lactose overloading event was to determine if the ion exchange fiber can work in a long term. Both of these two organic overloading evernts last eight days and applied three times higher lactose concentration than steady state. As seen in Figure 3.9, the reactor with IXF survived from the organic overload event again. So the Fiban X-1 can be used in a long term.

3.2.5 The comparison of organic overload fourth and fifth experiment

The comparison of organic overload fourth and fifth experiment was used to show that how the initial lactose concentration impacted the performance of anaerobic reactors during organic overload event. The Figure 3.10 showed the result of comparison between the fourth and the fifth organic overloading experiments. These two experiments were running under almost the same experiment condition except the influent lactose concentration and period of overload event, In the fifth experiment, its lactose concentration was 20 g/L, instead of 15 g/L and compared with 8 days in fourth experiment, its organic overloading event lasted 5 days. Both of them were applied to three times higher lactose concentration after the steady states reached. as seen in Figure 3.10, the accumulated methane production at day 5 for the fourth experiment reactors were much higher than the same types of reactors in the fifth experiment, even if much more lactose was added into the fifth experiment reactors. That can be attributed to the much smaller inhihibition to mathanogenic archaea in the higher pH solution. So unlike the generated methane at steady states, during the organic overload, the methane generation can be affected not only the organic loading rate, but also pH. after five days' organic overloading event, all of reactors including the reactor with IXF fails(no methane generation) in the fifth experiment.On the contray, until the eighth day of the fourth organic overloading event, the reactor with

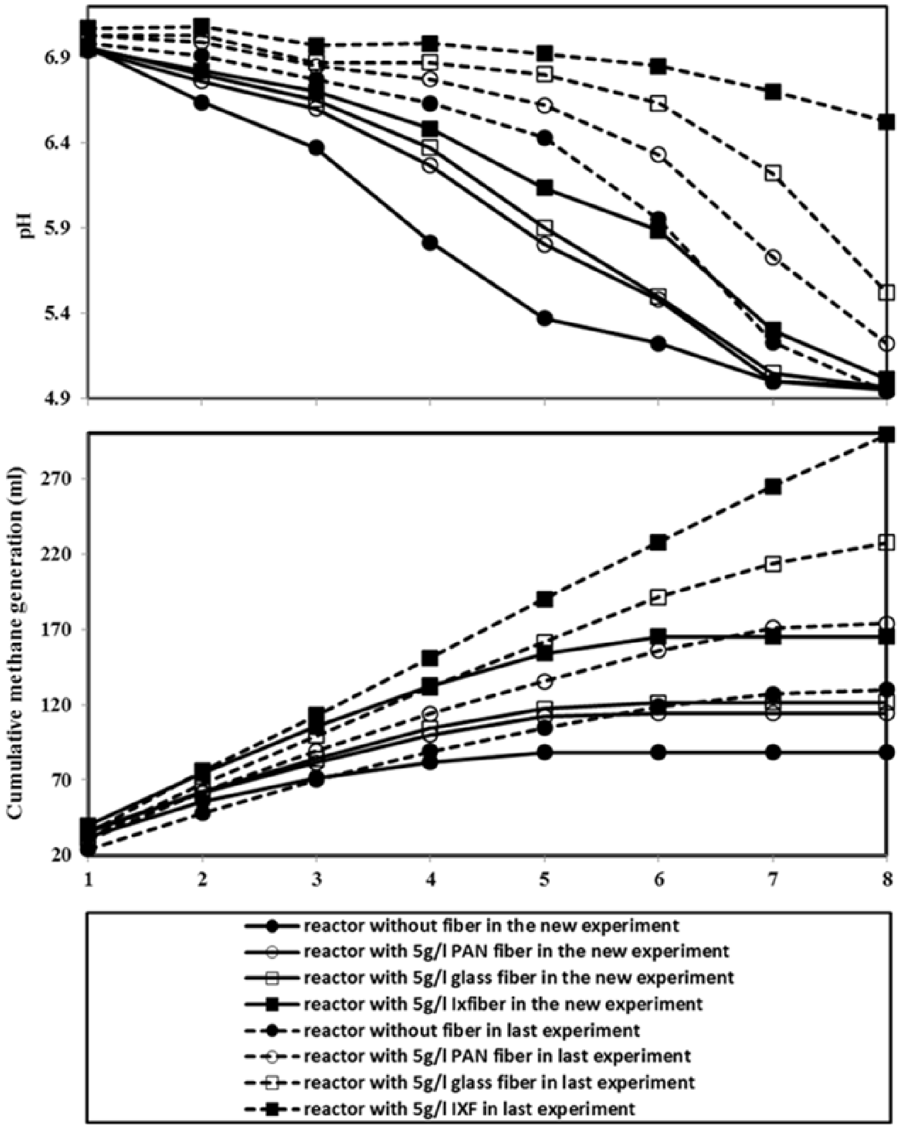

Figure 3.10 The comparison of the change of pH and methane production between two similar experiments in response to organic overloading events.

IXF was still working well. The pH of the fourth experiment at day seven should be similar to the pH of the fifth experiment at day 5 if methanogenic archaea doesn't work in the reactor. But In fact, the pH of reactors in the fourth experiment were higher the fifth one. That's because the higher pH increased the activity of methanogenic archaea and consequently increased methane generation and reduced the COD concentration. Then the reduced COD concentration was just able to prompte pH to increase. So these series of positive feedbacks caused the phenomena that even reactors with small different COD influent concentration may have large difference in pH and methane generation during the organic overloading event. This indicates that the initial nutrient condition can affect the mass of IXF for keeping anaerobic reactors against organic overloads.

3.2.6 The relationship among normalized methane generation, pH and COD

When anaerobic reactors met the excessive organic matters, two factors can affect the methane production. One was positive effect, which more food caused higher bacteria specific growth rate and then increased methane production. The other one was negative effect, which low pH and high COD would inhibit methane generation. For example, the pH can affect reactor's methane generation through affecting bacteria's enzyme acitivity.

So a close examination of the relationship between COD concentration, PH and methane generation rate from data of four organic overloading events was made (The second overload experiment use the variable time intervals, so its result was not presented in the Figure 3.11). As seen in Figure 3.11, the circles in the ellipse were the data under steady states. Others circles were the data during and after organic overloading event. The relationship between pH and normalized methane generation can be seperated into three part. Between pH ~7.0 to ~6.4, the reactors operated well and actively produced methane. Below a pH of ~6.0, the reactors became unstable and failed, with methane production decreasing rapidly as the pH dropped. The range between pH ~6.0 to ~6.4 was a transition region where the reactor operation fluctuated and they tended towards instability. So the conclusion was that when pH is above 6.4, the positive effect is more significant than the negative effect. When pH is below 6.0, the conclusion was just opposite.

A examination of COD mainly composed of acetate, propionate, butyrate and methane generation found that in each organic overload experiment, for example in Figures 3.8 and 3.9, the reactor with higher methane generation always had a lower COD concentration. However, after all of different experiments' data was put together, it was clear to see that the methane generation was not a function of COD concentation when COD was over 2 g/L. As seen in Figure 3.11, the normalized methane generation can decreased to zero not only when COD concentration was above 6 g/L, but also when it was between 2 g/L and 4 g/L, regardless of some data

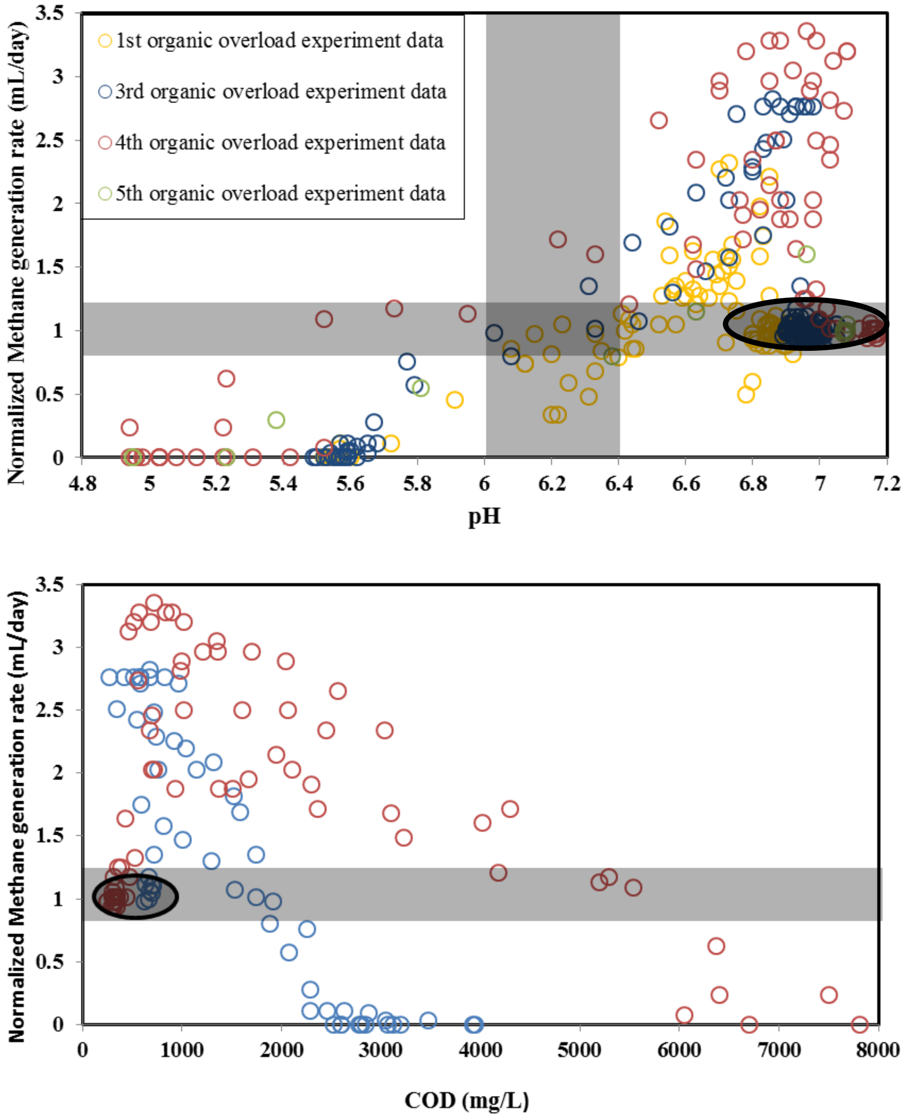

Figure 3.11 The relationship between Normalized methane generation and pH or COD. The normalized methane generation was calculated through using V/(V$_{average}$ at steady state).

circles located in the positive effective region in this COD range. And even reactors in the COD range between 4 g/L and 6 g/L had higher normalized methane generation than reactors in the COD range between 2 g/L and 4 g/L. The most significant difference between the pH and COD figures was that in the pH figure, all of data circles have the same spread pattern regarless of different experiment conditions. Even if in the single COD experiment, the reactor with higher methane generation always had a lower COD concentration, however, the two data sets cannot be fused into a pattern. So it is concluded that VFA may be not an "independent" toxic substance like pH, because the fused data sets didn't give us a clear "restricted zone" of COD concentration. Its toxicity can be related to pH, biomass, HRT and so on.

3.3 Demonstration of heavy metal's toxicity prevention with iminodiacetate IXF.

3.3.1 The first result of Nickel overload experiment

Results from the nickel shock-loading experiment was shown in Figure 3.12. The addition of nickel on day 42.5 resulted in an immediate decline in methane production in next two days. And at that time the reactor's pH is not lower enough to cause such a severe methane generation inhibition. So the sharp decreased methane generation should be mainly caused by the toxicity of nickel. After day 44.5, both of the reactors without fiber and with IXF's methane generation became fluctuated. But obviously the methane generation of reactor with IXF had a upward trend and the reactor

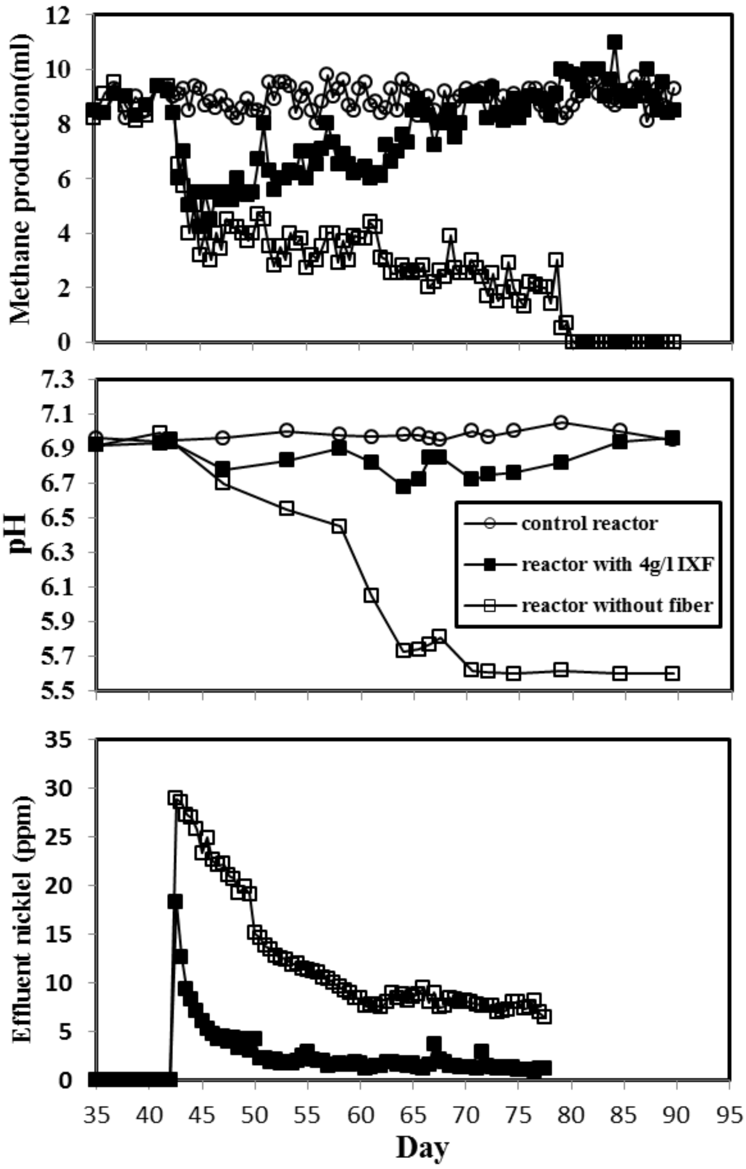

Figure 3.12 Demonstration of nickel toxicity prevention with Fiban X-1

without fiber had a downward trend because the reactor with IXF had a the significant decreased nickel concentration. At the same time, its pH was still decreasing because it's generally believed that the acidogensis bacteria was much more resistant to the toxicity of heacy metals than methanogenic archaea.[64] In addtion, studies commonly showed if pH was lower than 6.5, the inhibition effect of pH began to work and pH can be a critical inhibition factor if it is lower than 6.0. In this case, the performance of reactor without fiber did not recover to its steady state before pH dropprd below than 6.5 or even 6.0. Because of the inhibition of low pH and metal toxicity, finally the reactor's methane generation dropped to zero and pH decrease to 5.6. Generally speaking, heavy metals can affact the reactors stability, but most of cases showed that the reactors were able to recover through acclimating to heavy metals.[65,66] But in this case, it showed that the event of nickel overload can induce the drop of pH and consequently both of low pH inhibition and nickel toxicity can make anaerobic reactors lose their perfomance completedly. Especially, when pH is lower than 6.0, the low pH alone could lead the reactors to fail.

The reactor with IXF showed a better performance than the reactor without fiber including higher methane generation, higher pH and lower nickel concentration. That was because that the ion exchange capacity of Fiban X-1 helped reactors to relieve the toxicity of nickel. For example, the nickel concentrations of reactors without fiber, with 4 g/L fiber were 29 mg/L and 18.2 mg/L at the last day of adding chromate (day 42.5). And at day 50.5, the nickel conentrations of both of reactors with IXF were around 2 mg/L and did not show major changes over the next month. For the reactor without IXF at day 50.5, its concentration was 14.68 mg/L.

3.3.2 The second exepriment of nickel overloading experiment

Results from the second nickel shock-loading experiments are shown in Figure 3.13. Since the attached bacteria was a concern to improve the performance of anaerobic media,[61] so in this experiment the glass fiber without ion exchange capacity was also added into anaerobic reactor and it was compared with the performance of anaerbic reactors with IXF. Nickel was added into reactors at day 31, 33 and 34. At days 31, only the reactors with 2 g/L IXF and with 8 g/L glassfiber had a slightly decreased methane generation. But at day 32, these two reactors' methane generation returned to its normal level. In order to make differences among those reactors, nickel was added into reactors in day 33 and 34. Due to the consecutive nickel addition at day 33, and 34, the methane generation of the reactors without fiber and with glassfiber, have the sharp decreased methane generation. Their methane generations were decreased to 2.8 and 3.0 ml. After day 34, addition of nickel was stopped and then their methane generation became fluctuated from 1.8 to 3.5 mL/day and from 2 to 3 mL/day respectively. Both of the reactors with IXF showed a better performance than the reactor without IXF including higher methane generation and lower aqueous Ni concentration. That was because the ion exchange capacity of Fiban X-1 helped reactors to relieve the toxicity of nickel. For example, the nickel concentrations of

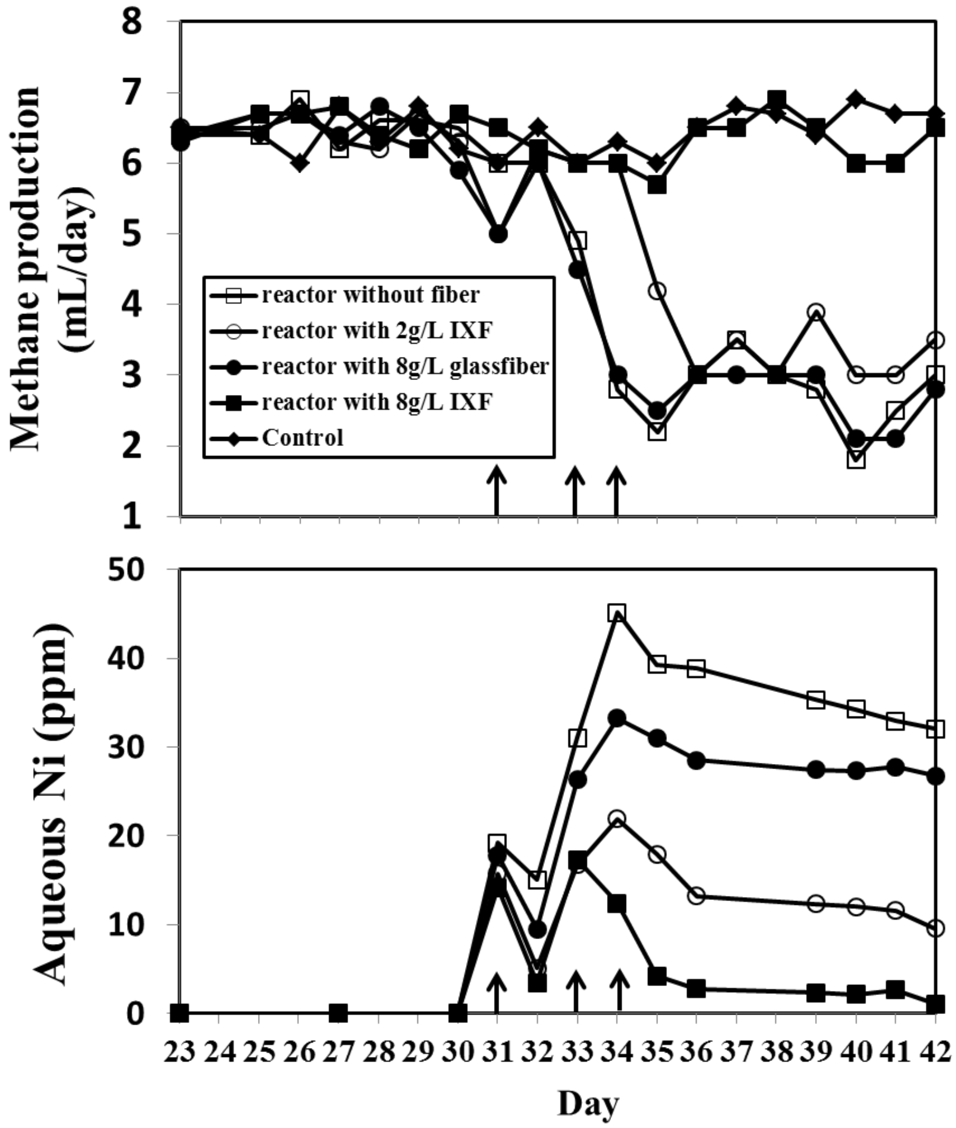

Figure 3.13 Comparison of the performance of reactor with IXF and with glass fiber in the nickel overload experiment

reactors without fiber, with 8g/L glass-fiber with 2 g/L IXF fiber and 8 g/L IXF fiber were 45.18 mg/L, 33.26 mg/L, 21.88 mg/L and 12.40 mg/L at the end of nickel addition day 34. And at day 36, the Ni conentration of reactors with 8 g/L IXF and 2 g/L IXF were around 2.80 mg/L and 13.22 mg/L, respectively, and their aqueous concentrations did not show major changes in the next week. For the reactor without fiber and with 8 g/L glass fiber, their aqueous Ni concentrations were higher than the other reactors. And even at the last day of this experiment (day 42), their Ni concentrations were still higher than 20 mg/L.

The performance of the reactor with 8 g/L IXF was the best among all types of reactors. Compared with the reactor with 8 g/L IXF, the addition of nickel began to affect the methane generation of reactor with 2 g/L IXF at day 35. Its methane generation decreased from normal level to 4.2 mL/day at day 35. And in the next week, its methane generation didn't recover.

Commonly, there is not a coherent answer about the inhition concentration of Nickel.[29,35-37,41,47] But in each overload experiment, the higher the nickel concentration, the severer the inhibition does.[34-36] In this experiment, the high solubility of nickel provided the chance for us to see the significant nickel concentation difference between the reactors with IXF fiber and without IXF fiber.

3.3.3 The first result of copper overload experiment

As seen in the Figure 3.13, the addition of glass fiber didn’t help anaerobic reactor to improve methane generation. However, this result didn’t happen to all of heavy metals’ overloading event. For example in the Figure 3.14, the copper was added into reactors at day 34 and 36. The copper overload experiment showed that the reactor with 6 g/L glassfiber has a high methane generation than the reactor with 1.5 g/L IXF. Even compared with the reactor with 6 g/L IXF, the methane generation of reactor with glass fiber was just slightly lower at day 34 and 35. Here this observation can be attributed to that bacteria biofilm had diverse resistances to different heavy metals.[61,62]

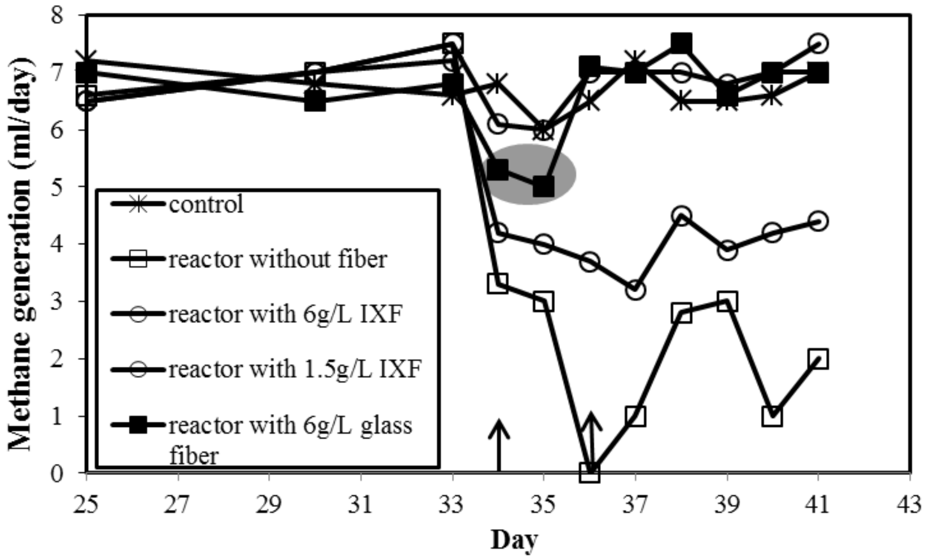

Figure 3.14 The effect of the addition of glass fiber in the copper overload experiment.

3.3.4 The result of chromate overloading experiment

Results from the chromate shock-loading experiments were shown in Figure 3.15. The addition of chromate on Days 32, 33 and 34 resulted in an immediate decline in methane production from normal level to zero for the reactor without fiber. After day 34, the addition of chromate was stopped and reactor without fiber's methane generation became fluctuated from 0 to 4.4 mL because of the significant decreased aquoues chromate concentration, but its methane generation was still lower than its steady state value. At the same time, its pH was still decreasing because it's generally believed that the acidogensis bacteria was much more resistant to the toxicity of heacy metals than methanogenic archaea.[65] Again, studies showed pH can be a critical inhibition factor if it is lower than 6.0. In this case, the performance of reactor without fiber did not recover to its steady state before pH dropprd below 6.0. Because of the inhibition of low pH and metal toxicity, finally the reactor's methane generation dropped to zero and pH decrease to 5.44.

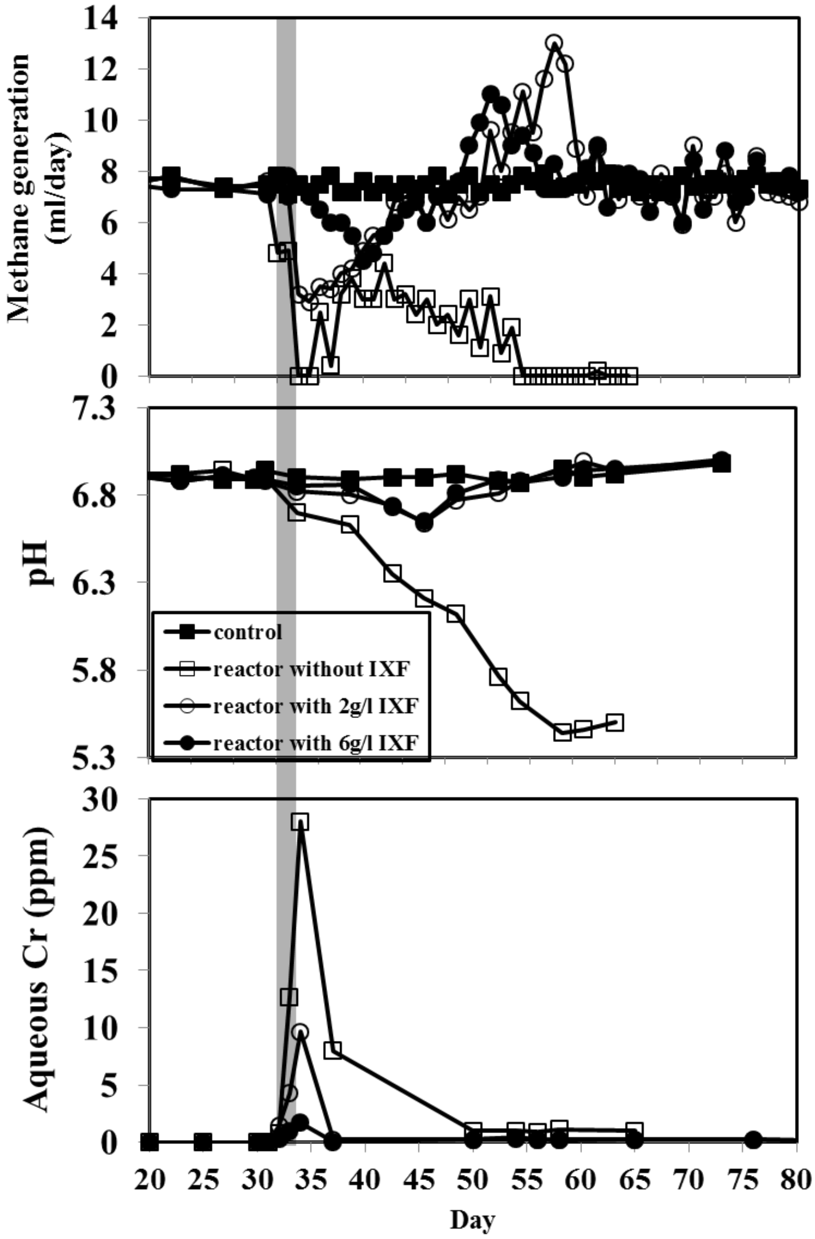

Figure 3.15 Results of the chromate shock-loading experiment.

Both of the reactors with IXF showed a better performance than the reactor without fiber including higher methane generation, higher pH and lower aqueous chromate concentration. That's because that the ion exchange capacity of Fiban A-1 helped reactors to relieve the toxicity of chromate. For example, the chromate concentration of reactors without fiber, with 2 g/L fiber and 6 g/L fiber were 28 mg/L, 9.64 mg/L and 1.7 mg/L at the last day of chromate addition (day 34). And at day 37, the Cr conentration of both of reactors with IXF were around 0.2 mg/L and did not show significant change in the next month. For the reactor without IXF at day 37, its concentration was 7.97 mg/L and after two weeks, its Cr concentration is still 1 mg/L and kept this concentration from day 50 to 65.

Both of the two reactors with IXF had the pH decreased down to approximately 6.65 from day 34 to 46. And they had similar pH recover curve, so the effect of pH inhibition difference between these two reactors can be excluded. Compared with the reactor with 2 g/L IXF, the reactor with 6 g/L IXF had a much slower rate of decreased methane generation rate. Its methane generation decreased from normal level to 4.4 mL/day from day 35 to 40, but the methane generation of reactor containing 2 g/L IXF decreased from normal level to 2.9 mL/day in two days. There were two peaks during the recovery of methane generation, that was caused by the consumption of accumulated fatty acids in the recovery process.

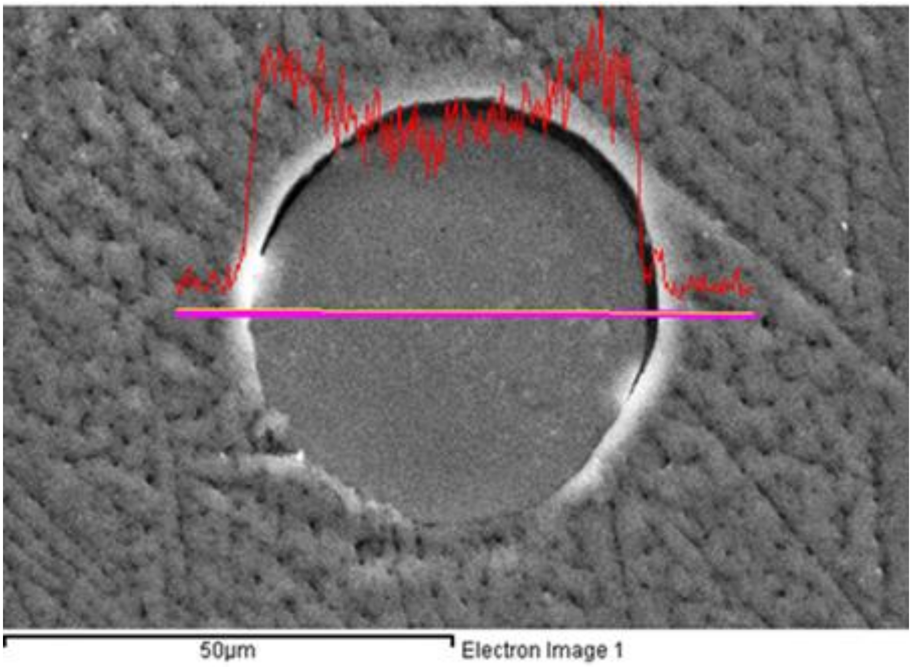

Figure 3.16 Chromate distribution along the diameter of Fiban A-1

After the biological experiment, the element-chromium of Fiban A-1 was detected by EDX. As seen in the Figure 3.16, the chromium concentration was drawn along the diameter of Fiban A-1. Because the chromate diffused into fiber from surrounding to center, the chromium concentration in the outer ring of fiber was higher than that in the center. So the red chromium line presented a “V” curve along the diameter of Fiban X-1. And at the same time, it demonstrated the capacity of this fiber was not used up in this chromate overload experiment.

3.3.5 The result of second copper overload experiment

A 3- month experiment result didn’t show that Mg and Ca can replace Ni or Cu without pH change, which demonstrated the Fiban X-1 may not achieve self-regeneration after heavy metals overloading events in a short time. Fortunately, anaerobic bacteria can adapt to heavy metals environment very well, if they have survived from the similar event since the recent period, which is shown in Figure 3.17. Here, 8 mg of $CuCl_2 \cdot 2H_2O$ (47 mmole) was added to 100 mL anaerobic reactors at days 32, 33 and 93,94. This experiment result was very similar to the result of nickel and chromate experiment. Because of the inhibition of low pH and metal toxicity, finally the reactor without fiber’s methane generation dropped to zero and pH decrease to 5.68. And both of the reactors with IXF survived from the copper overload event with the help of ion exchange fiber and always showed better

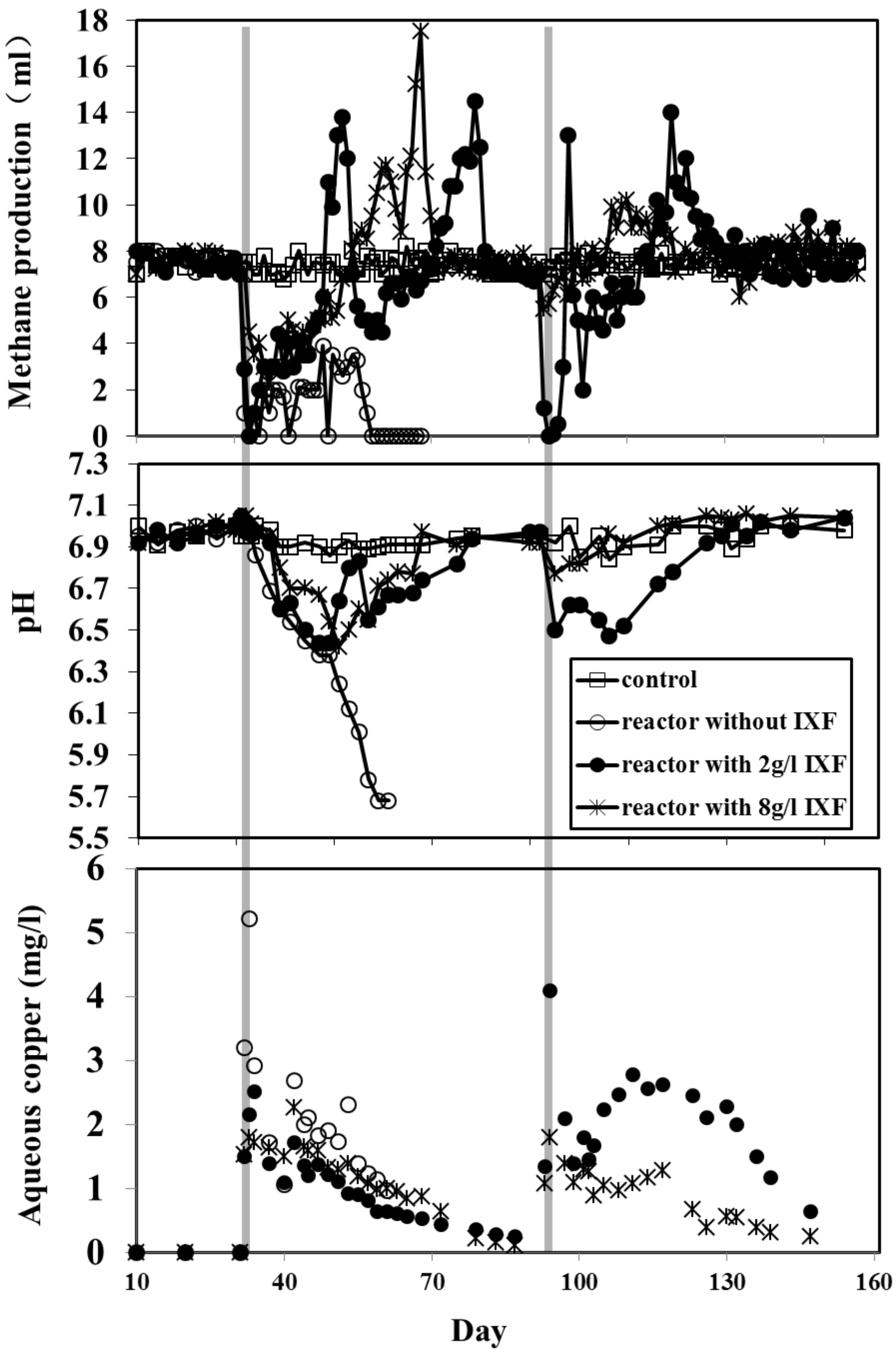

Figure 3.17 Demonstration of adaptation to copper toxicity by anaerobic reactor.

performance than the reactor without fiber including higher methane generation, higher pH and lower aqueous Cu concentration. During the second copper overload event, both of the reactors with IXF showed better performance than their performance in the first event, even if the aqueous copper concentration was higher than it in the first event. For example, in the first cycle, the days of methane generation and pH recovery for reactors with 2 g/L IXF are 49 days and 19 days, respectively. However, in the second cycle, it just took 39 days and 10 days for their recovery. Similarly, for the reactor with 8 g/L IXF, the time in the first cycle is 38 days and 16 days. But in the second cycle, they were just 28 days and 4 days. This result has fully demonstrated that anaerobic bacteria can adapt to heavy metals environment very well when they meet multiple heavy metal overloading events.

3.4 Aging of Fiban X-1

As seen in Figure 3.18, an experiment result showed in response to the addition of 30 mg $NiCl_2.6H_20$, the fresh, 4-month, 8-month, 1-year use IXF presented 1.1755, 1.1528, 1.1341, 1.1135 meq-Ni^{2+}/g-fiber capacity. It just slightly decreased as the increased use time, which demonstrated the Fiban X-1 can be used for a long term biological experiment without significant capacity loss.

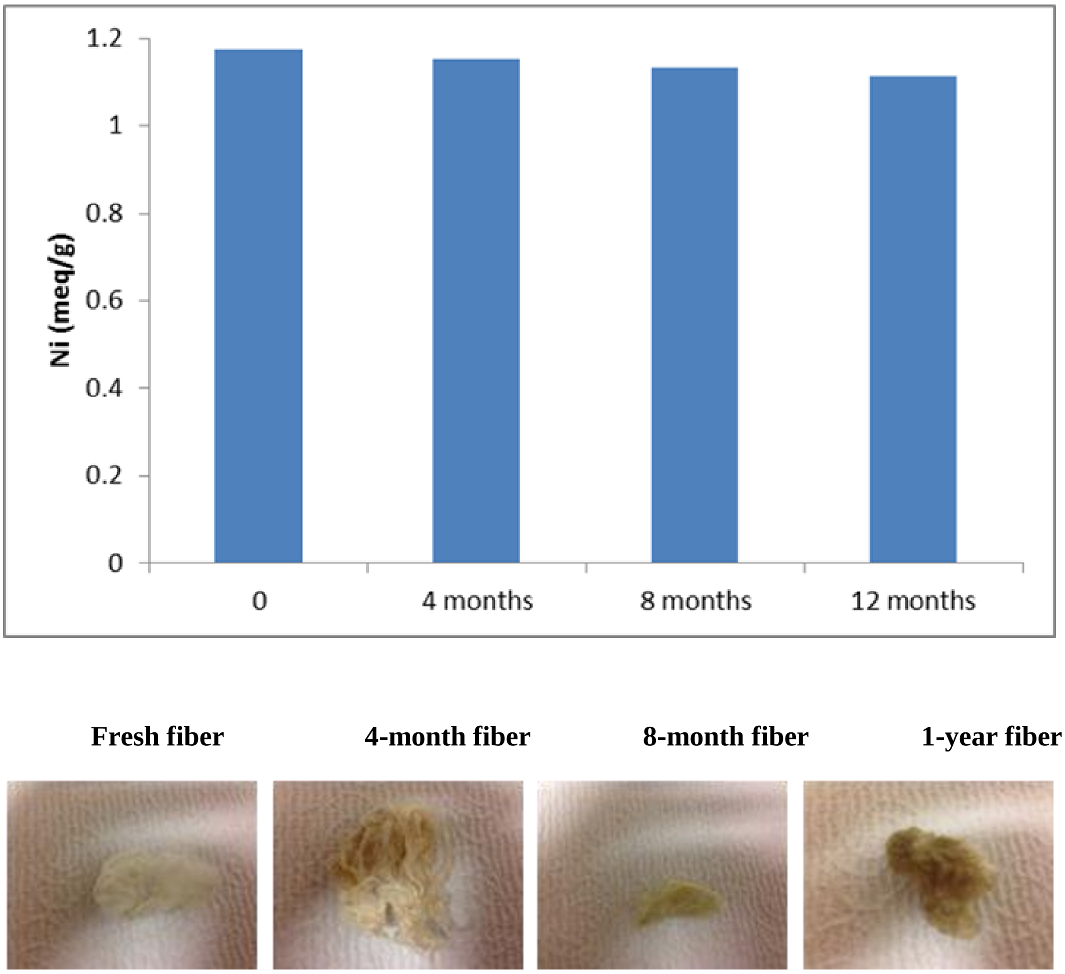

Figure 3.18 Aging of ion exchange fiber.

4. DEVELOPMENT OF THE MODEL OF METHANE GENERATION IN ANAEROBIC REACTORS

4.1 The model of methane generation

Anaerobic digestion is a complex microbiological process and it involves three main steps:

1. The hydrolysis of the complex organic matter, for example, cellulose will be decomposed into soluble sugars

2. The acidogenic process, which causes the production of volatile fatty acids,

3. The methanogenic process, which methanogenic archaea convert the volatile acids into the final products, including carbon dioxide and methane.

Acetic acid is the most important precursor of methane among all kinds of volatile fatty acids (VFA). Because methanogenic archaea have the lowest growth rate, methanogenesis process has been usually considered to be the rate-limiting step in modelling work.[67-72] The previous experiments have demonstrated the anaerobic reactors can be inhibited by the addition of excessive organic matters and heavy metals, so it is important to develop of new control model in order to avoid process failure. Since this study's objectives focused on the demonstration of the feasibility of ion exchange fiber in the anaerobic reactor, here i provided the method to model a simple batch-feed reactor's methane generation through the use of the third organic overloading experiment data.

The equations used for modelling work

The model was developed for a batch-feed tank reactor, without recycling. The self-inhibition function is used to define the specific growth rate (μ), since it is well documented that both of pH and volatile fatty acids can inhibited methane generation:

$$\mu = \frac{\mu_{max}}{1+\frac{HS}{K_i}+\frac{K_S}{HS}} \qquad \text{Equation 4.1}$$

The above inhibition function assumes that the un-ionised volatile acids are the inhibition agents and they are also the substrates for methanogenic archaea growth.[74] From the acetic acid equilibrium constant:

$$K_a = \frac{S^- \times H^+}{HS} \qquad \text{Equation 4.2}$$

And the total acetic acid concentration is equal to:

$$S_T = HS + S^- \qquad \text{Equation 4.3}$$

This self-inhibition function can be re-written as a function of total acetic acid concentration:

$$\mu = \frac{\mu_{max}}{1+\frac{K_S \times (K_a+H^+)}{S_T \times H^+}+\frac{S_T \times H^+}{(K_a+H^+) \times K_i}} \qquad \text{Equation 4.4}$$

Where

μ = micro-organisms specific growth rate (d^{-1})

μ_{max} = maximum specific growth rate (d^{-1})

HS = un-ionised substrate concentration (mg/l)

K_s = saturation constant (mg/l)

K_i = substrate inhibition constant (mg/l)

K_a = acetic acid equilibrium constant

S^- = ionised substrate concentration (mg/l)

H^+ = hydrogen ion concentration (mg/l)

S_T = total substrate concentration in the reactor (mg/l)

The model developed thus far assumes the following:

1. methanogenesis is assumed as the limiting step;
2. substrate is only acetic acid;
3. the mass of methanogenic micro-organisms is not changed through the organic overloading event for the simplest model (Δ X = 0).

So, the volume of generated methane per feed can be written as follows:

$$V_{methane} = \left(\left(COD_{n-\frac{1}{b}} + COD_{in} - COD_n\right) \times (1 - f_c) \ - \frac{1}{b} \times \frac{f_s}{Y} \times X \frac{\mu_{max}}{1 + \frac{K_S \times (K_a + H^+)}{S_T \times H^+} + \frac{S_T \times H^+}{(K_a + H^+) \times K_i}}\right) \times F_{\frac{V}{M}} \ \times \frac{T_{308}}{T_{273}} \times I \times \mathrm{V}_{reactor}$$

Equation 4.5

The term $\left(COD_{n-1/b} + COD_{in} - COD_n\right) \times (1 - f_c)$ represents the COD concentration which is available for methanogenic archaea per feed. The term $\frac{1}{b} \times \frac{f_s}{Y} \times X \frac{\mu_{max}}{1+\frac{K_S \times (K_a + H^+)}{S_T \times H^+} + \frac{S_T \times H^+}{(K_a + H^+) \times K_i}}$ represents the COD concentration used by methanogenic archaea for cellular synthesis.

Here

$COD_{n-1/b}$= reactor's COD concentration at day n-1/b (mg/l)

COD_n= reactor's COD concentration at day n (mg/l)

COD_{in}= COD concentration in the influent (mg/l)

Y = yield coefficient, mass of micro-organisms produced per mass of substrate utilized (g g-1).

X = micro-organism concentration in the reactor (mg/l)

θ = hydraulic retention time (d)

f_c= the fraction of carbon used by acetogens

$F_{(V/M)}$= the theoretical methane production ration= 0.35 m3/kg-COD78

fs= the fraction of carbon used by methanogens

I = inhibition factor, related to pH[60]

I = 1 when pH is above 6.5

$I = \exp\left(\frac{-3 \times (pH - pH_{ul})^2}{(pH - pH_{ll})^2}\right)$ when pH is below 6.5

b= feeding times per day

$pH_{uL=}$ the pH points at which the organisms are not inhibited

$Ph_{LL=}$ the pH points at which the inhibition is completed

The reported kinetic parameters are provided in Table 4.1. And the first line parameters in Table 4.1 are used in this model: $\mu_{max} = 0.4\ d^{-l}$, $K_s = 2\ mg\ l^{-1}$, $K_i = 40\ mg\ l^{-1}$ $Y = 0.05\ g\ g^{-1}$,

The carbon can be used by both of acetogens and methanogens. Here I use $f_c = 0.22$, i.e., 22% of the carbon was used by the acetogens, and obtain $pH_{uL=6.5}$ and $pH_{LL=5.5}$ from Figure 3.11.

The model was applied into the third organic overloading experiment and example results are presented in Figure 4.1. As seen in this figure, the modelling curve represented the experiment data well.

Table 4.1 Kinetics parameters for aceticlastic methanogensis.

U_{max}(day^{-1})	K_s (mg-COD/L)	Y	K_i (mg-COD/L)	Substrate	Ref.
0.4	2	0.05	40	Acetate	73
0.474	930	0.07	-	Acetate	74
0.1	28	0.03	-	Acetate	74
1.406	930	0.07	-	Acetate	75
0.047	11	0.01	-	Acetate	75
0.41	35	0.06	-	glucose	76
0.298	213	0.03	-	Acetate	77

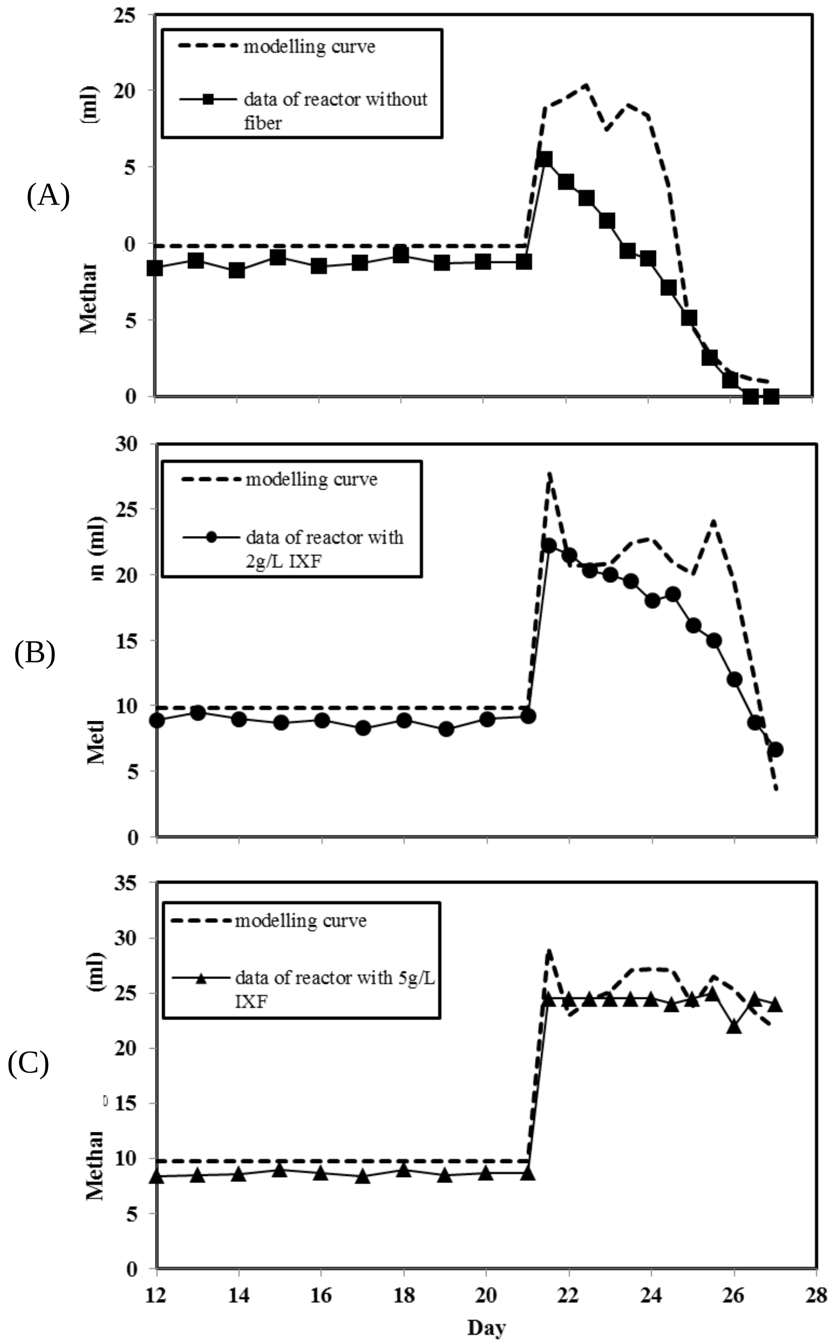

Figure 4.1 The comparison of methane generation of experiment data and its modelling curve. Figure A,B,C represented the modelling curves and data of reactor without fiber, with 2g/L IXF and 5g/L IXF respectively.

4.2 Glossary

μ = micro-organisms specific growth rate (d^{-l})

μ_{max} = maximum specific growth rate (d^{-1})

HS = un-ionised substrate concentration (mg/l)

K_s = saturation constant (mg/l)

K_i = substrate inhibition constant (mg/l)

K_a = acetic acid equilibrium constant

S^- = ionised substrate concentration (mg/l)

H^+ = hydrogen ion concentration (mg/l)

S_T = total substrate concentration in the reactor (mg/l)

COD_{n-1}= reactor's COD concentration at day n-1 (mg/l)

COD_n= reactor's COD concentration at day n (mg/l)

COD_{in}= COD concentration in the influent (mg/l)

Y = yield coefficient, mass of micro-organisms produced per mass of substrate utilized (g g-1).

X = micro-organism concentration in the reactor (mg/l)

θ = hydraulic retention time (d)

f_c= the fraction of carbon used by acetogens

$F_{(V/M)}=$ the theoretically methane production ration= 0.35 M3/kg-COD[78]

fs= the fraction of carbon used by methanogens

I = inhibition factor, related to pH[60]

$$I = 1 \qquad \text{when pH is above 6.5}$$

$$I = \exp\left(\frac{-3\times(pH-pH_{ul})^2}{(pH-pH_{ll})^2}\right) \qquad \text{when pH is below 6.5}$$

$pH_{uL}=$ the pH points at which the organisms are not inhibited

$Ph_{LL}=$ the pH points at which the inhibition is completed

b= feeding times per day

5. SUMMARY OF CONCLUSIONS AND RECOMMENDATION FOR FUTURE STUDIES

5.1 Conclusions

Low energy consumption, low sludge production and generation of methane as a usable biofuel are the main desirable advantages of anaerobic biological wastewater treatment process. However, despite these advantages, poor stability and susceptibility to failure have greatly hindered the applications of this attractive biological process. The concern about the process stability for treatment of industrial wastewater is that organic matters loading rates, heavy metals and alkalinity levels of the wastewater can have large variations which may cause severe stress conditions within the anaerobic reactors. The major reason for the failure of anaerobic biological processes is inhibition of the methanogenic archaea due to (a) the build-up of volatile acids causing lowering of pH and (b) toxic metals overloading of the reactor.

Here, I demonstrated that the ion exchange fiber is able to stabilize AD by buffering pH fluctuations and moderating shock-loads of dissolved toxic metals. Compared to other material or chemicals, it has high regeneration rate, reaction rate, ion selectivity and ion exchange capacity. Additionally, IXF is very stable to high alkaline solution and it can be easily retained inside the reactor.

Results provided positive data indicating that IXF can passively stabilize anaerobic reactors against organic overloading events and the addition of heavy metals including copper, nickel, and chromate. In the organic overloading experiments, the fiber without ion exchange capacity, including glass fiber and PAN fiber, can also benefit the methane generation by relating biofilm in the reactors but reactors with the same amount of IXF demonstrated much better performance than reactors with other fibers. Also, the relationships among the methane generation, pH and COD with four sets of data from organic overloading experiments were examined. The four sets of data followed the same pattern that between pH ~7.0 to ~6.4, the reactors operated well and actively produced methane. Below a pH of ~6.0, the reactors became unstable and failed, with methane production decreasing rapidly. The range between pH ~6.0 to ~6.4 was a transition region where the reactor operation fluctuated and they tended towards instability.

For experiments with the addition of heavy metals, the effluent metal concentration and methane generation demonstrated that the IXF helped reactors survived from heavy metals toxicity. In addition, results showed after exposure, reactors showed a significant pH drop and all of failed reactors had a low pH around 5.

Overall, the results indicated that the presence of the weak-acid IXF Fiban X-1 and strong-base IXF Fiban A-1 are capable of buffering anaerobic reactors to pH variations from variation in organic loading and can alleviate the toxicity of heavy metals. These results demonstrated the feasibility of using IXFs to passively buffer anaerobic reactors to upset and further development of this technology may make anaerobic treatment and energy recovery from industrial waste streams more attractive.

As shown in Table 5.1, 1 g Fiban X-1 is 192.5 times expensive than 1 g of sodium bicarbonate. Let's suppose that the life time of Fiban X-1 is 5 years and it's used in a biofilm reactor with an HRT of 1 day. Let's compare it to a reactor without fiber with an $NaHCO_3$ concentration maintained at 4g/L. The cost of $NaHCO_3$ per liter of reactor volume is (4 g/L/d)($0.4/kg-$NaHCO_3$)(5 yr)(365 d/yr) = $2.92/L. Because sodium bicarbonate's proton exchange capacity is seven times higher than Fiban X-1, the concentration of Fiban X-1 providing an equivalent exchange capacity is (4 g/L of $NaHCO_3$)(7 g-Fiban X-1/g-$NaHCO_3$) = 28 g/L. In the case, the cost of Fiban X-1 per liter of reactor is (28 g/L)($77/ kg)(0.001 kg/g) = $2.156/L. Thus, the cost of Fiban X-1 is $0.764/L cheaper than sodium bicarbonate.

In practice, fiber has additional advantages over buffer addition. For example, fibers don't require active monitoring and control, allowing minimal operator oversight when used to provide excess buffer capacity to alleviate organic overloading. In addition, the presence of IXF can also alleviate accidental inputs of heavy metals.

Table 5.1 The cost of various alkalinity chemicals

Chemical	Cost/tonne
CaO	$120
$NaOH$	$800
Na_2CO_3	$400
$NaHCO_3$	$400
NH_4OH	$800
MgO	$360
Fiban A-1	$123,000
Fiban X-1	$77,000
CIX[79]	$ 80,000

5.2 Recommendations

In this study, the main purpose is to demonstrate the feasibilty of ion exchange fiber for stabilizing anaerobic reactor. Basically, the 1 g IXF is 1/7 capacity of the same mass of sodium bicarbonate. The next study can mainly focus on developing the modelling work. The next student may develop a model through incorporating the demand of mass of IXF to the existed model of anaerobic biological wastewater treatment process.

It's necessary to explore more kinds of IXF, if possible. Espeically, for the organic overload experiments, the Fiban X-1 capacity is 0.6meq/g. This number is quite smaller than fiber's total exchange capacity. So fiber with larger proton ion exchange capacity can help the performance of anaerobic reactors better.

Finally, since the experiment data have provided positive data to demonstrate the IXF's feasibility in the anaerobic reactor, in future Pilot scale test with industrial waste with simulated waste flows is deserved to run.

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APPENDIX A

The investigation of proton adsorption capacity of Fiban X-1 in bacteria suspension was shown through SEM photos.

The experiment method is shown below:

100mL bacteria solution were mixed with 0.2 g cation ion exchange fiber in two reactors. After 24 hours mixing, one of reactor's pH was adjusted to 5.0 using HCl. The other reactor's pH was maintained at 7.0. Then after 24 hours mixing, the fibers in the two reactors were taken out and analysis using SEM-EDX. Seven sites on each fiber were analyzed for Na, Ca and Mg.

Conclusion:

For the fiber at pH 7, all sites showed strong Mg intensity while five sites showed moderate Ca intensity and no Na (Figures A.1 to A.7).

For the fiber at pH 5, only two sites showed weak Mg and Ca intensity and no Na (Figures A.8 to A.14).

These results demonstrated that H^+ is able to replace Mg^{2+} and Ca^{2+} in the fiber when inside anaerobic biological reactors.

Note: All SEM photos were taken under the same settings.

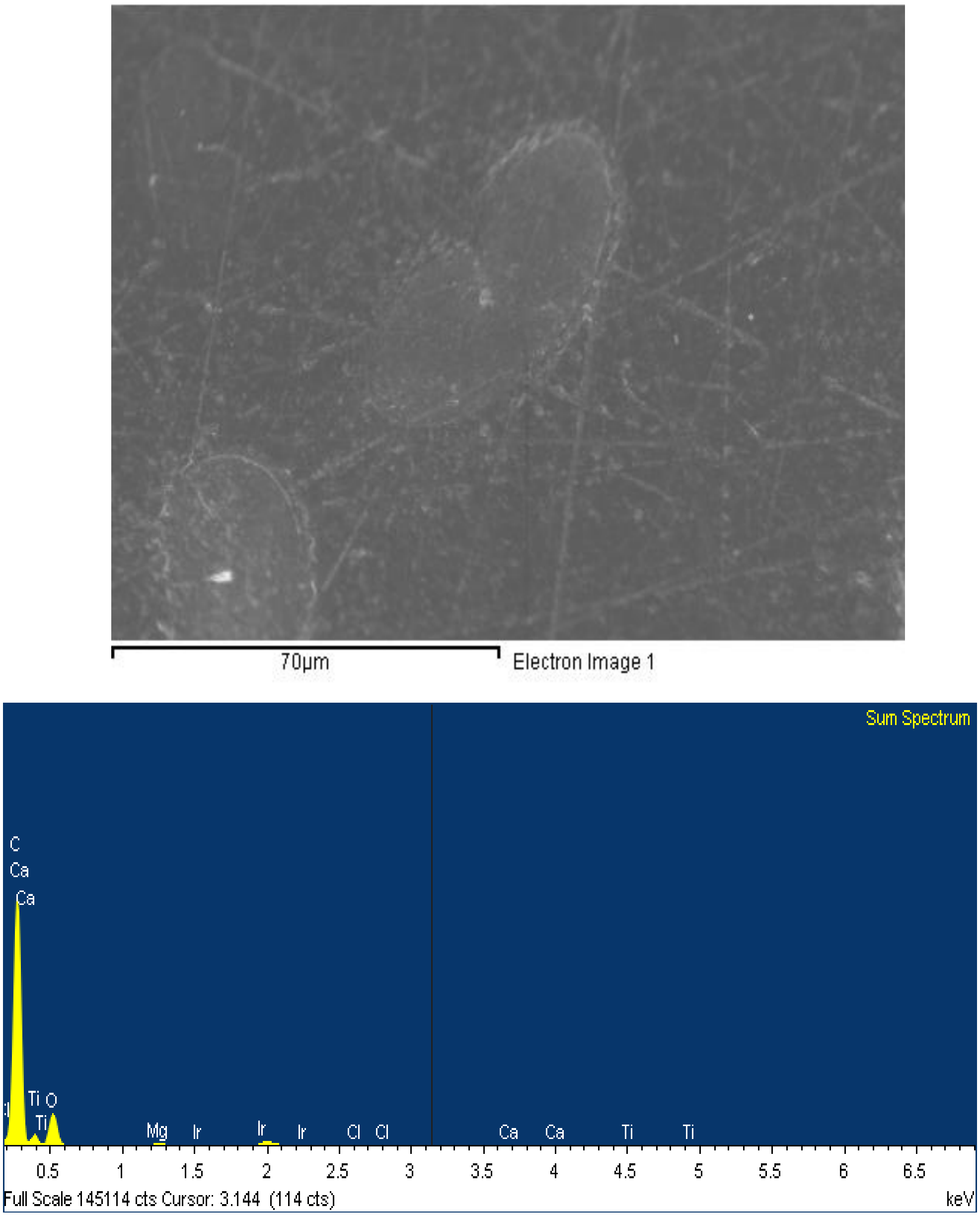
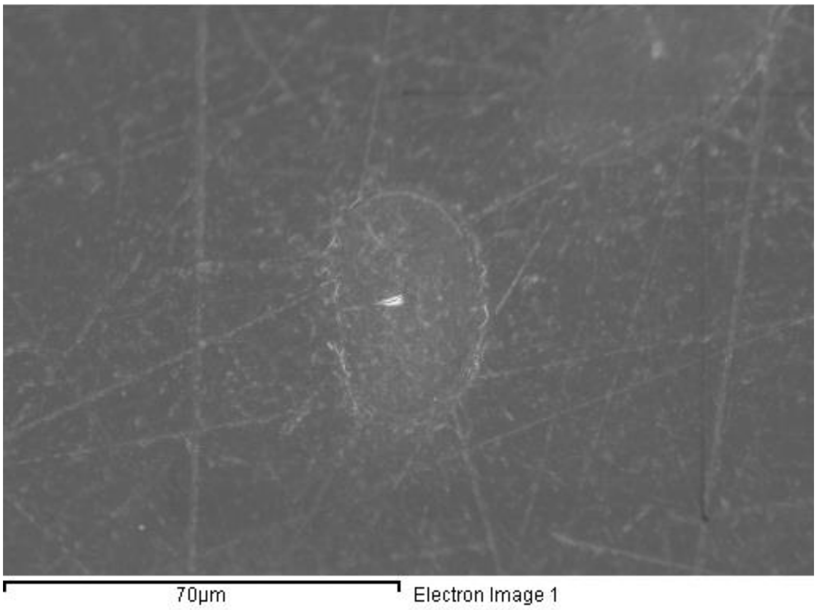


Figure A.1 The SEM photo of the cross section of a fiber and its elements spectrum. (pH = 7)



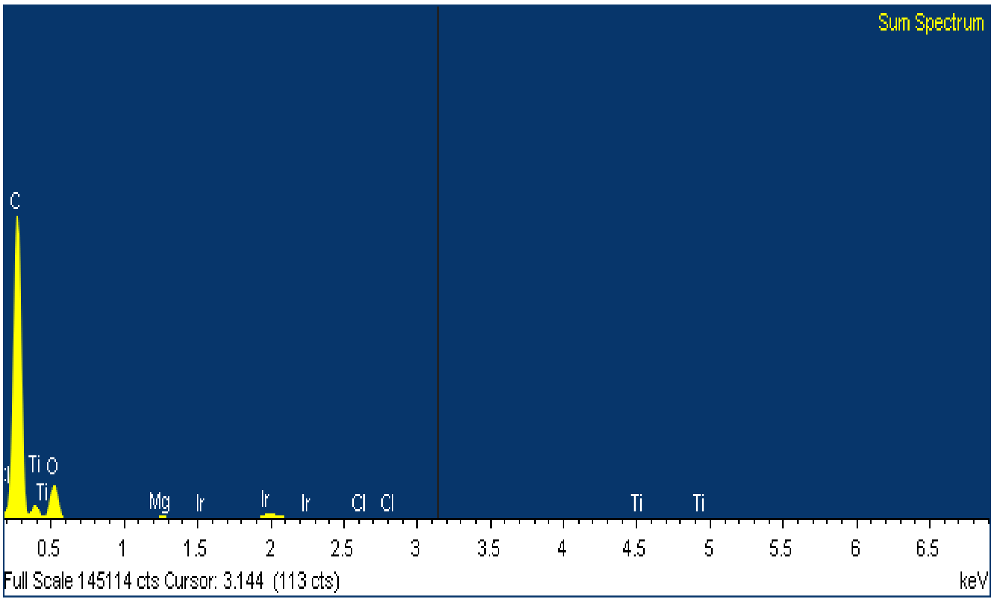


Figure A.2 The SEM photo of the cross section of a fiber and its elements spectrum. (pH = 7)

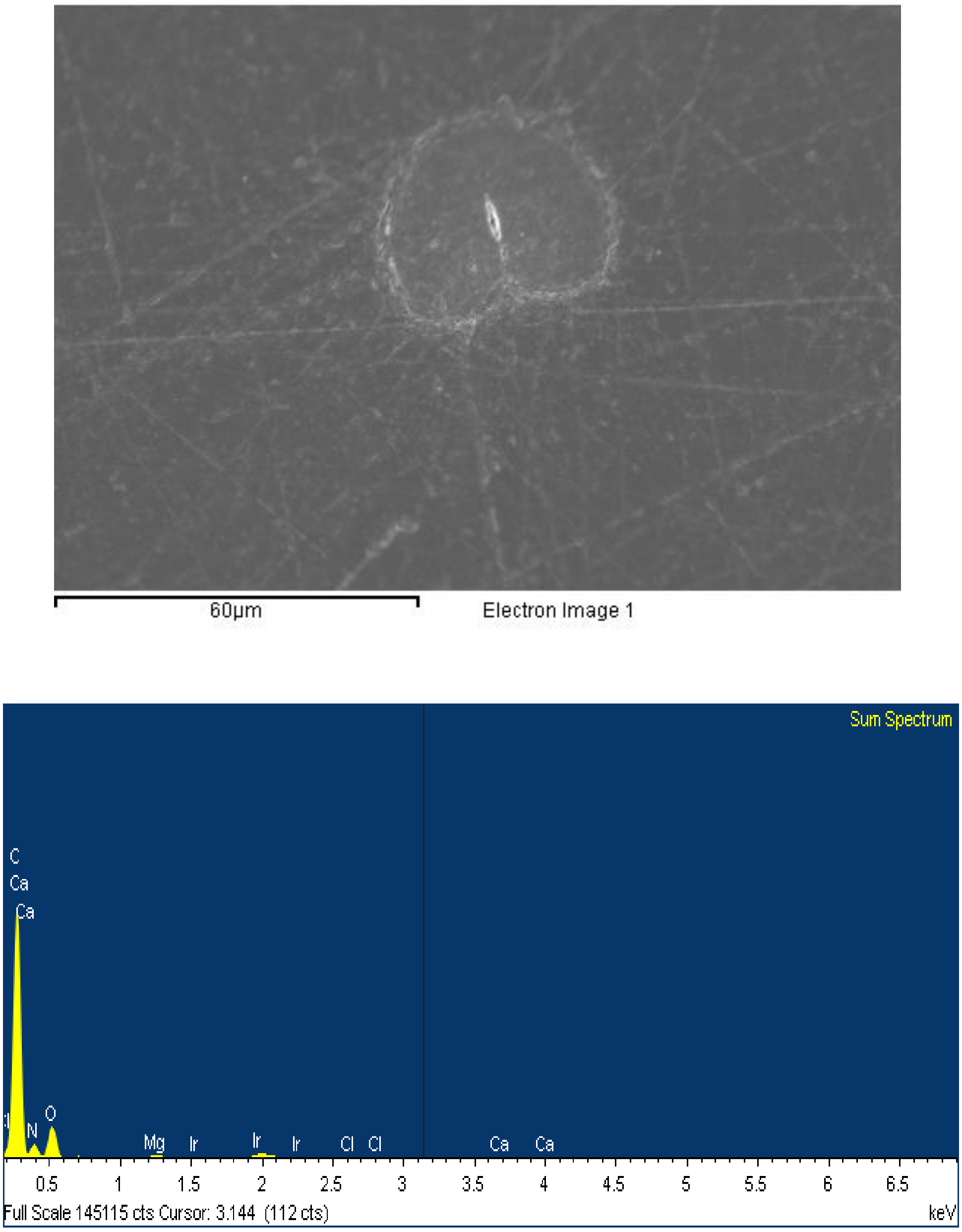
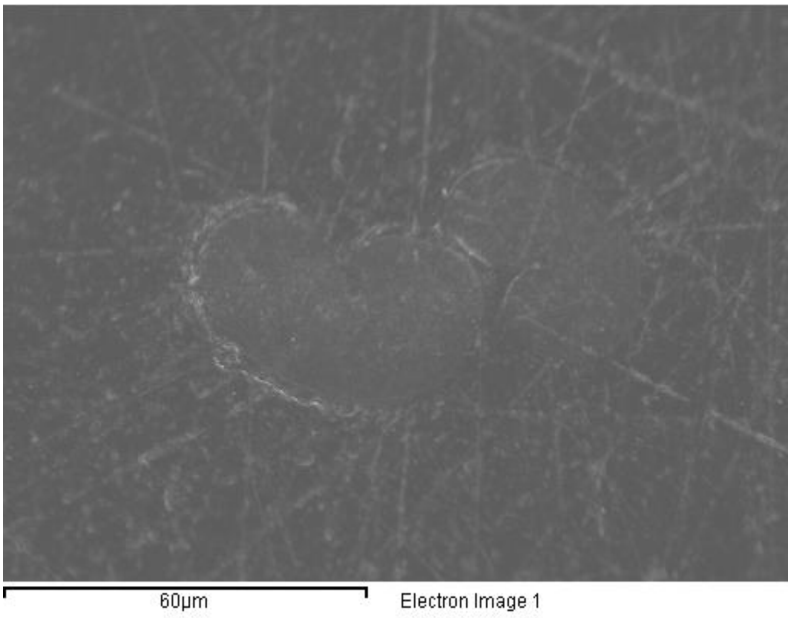


Figure A.3 The SEM photo of the cross section of a fiber and its elements spectrum. (pH = 7)



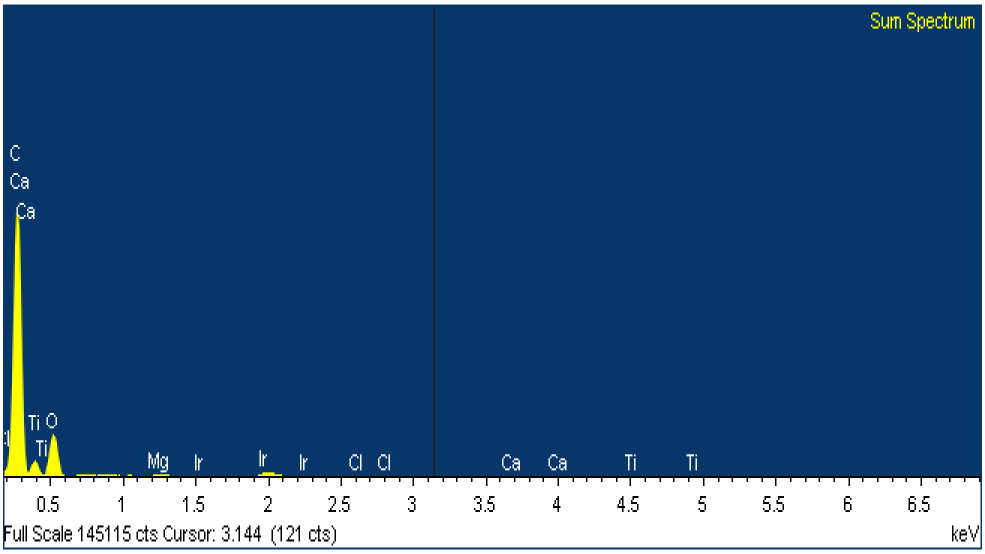
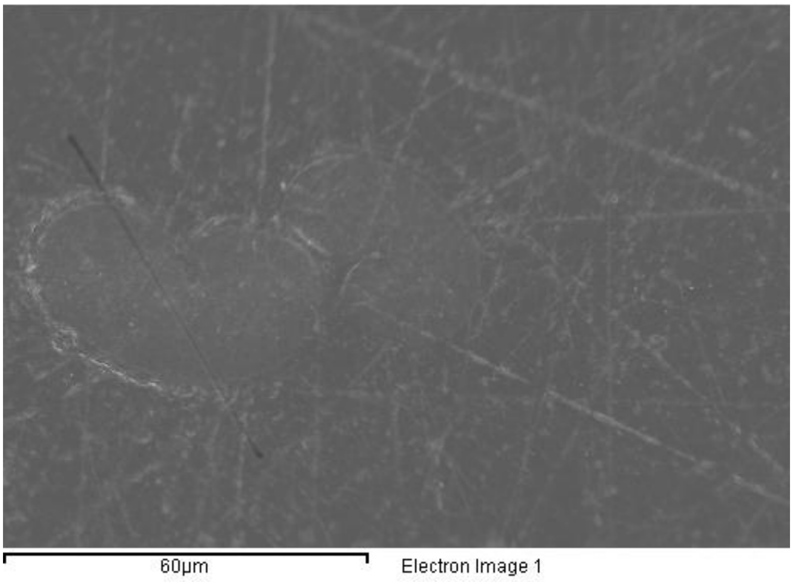


Figure A.4 The SEM photo of the cross section of a fiber and its elements spectrum. (pH = 7)



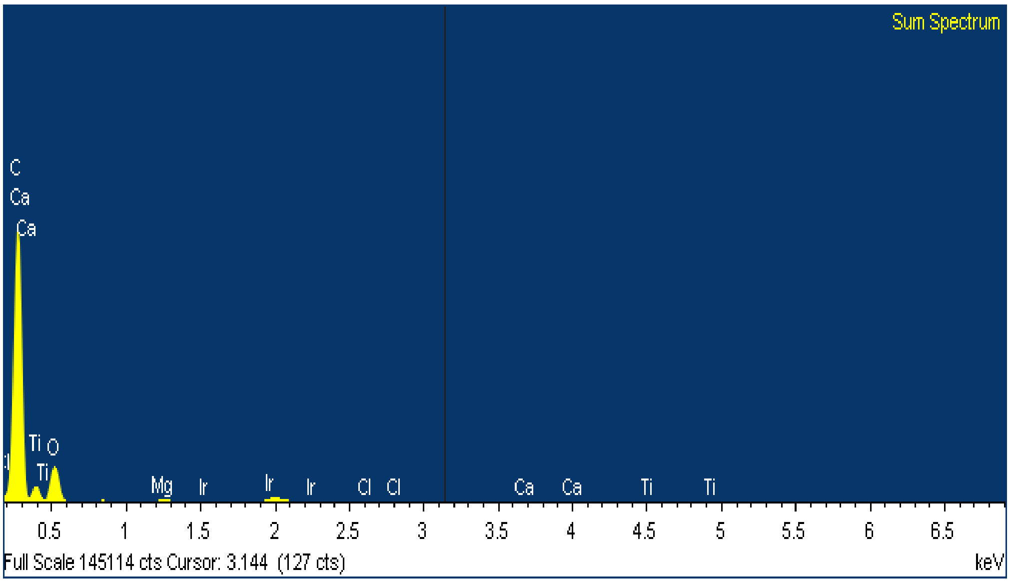
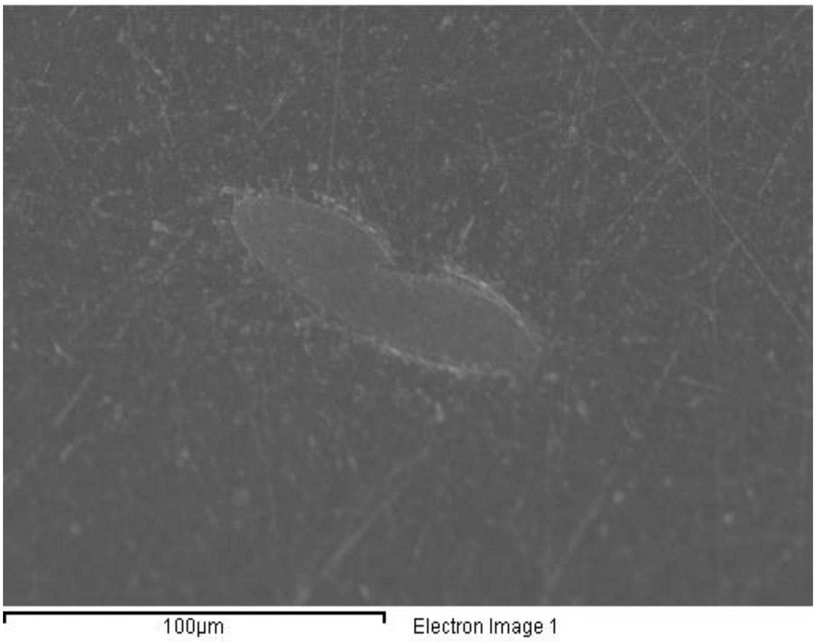


Figure A.5 The SEM photo of the cross section of a fiber and its elements spectrum. (pH = 7)



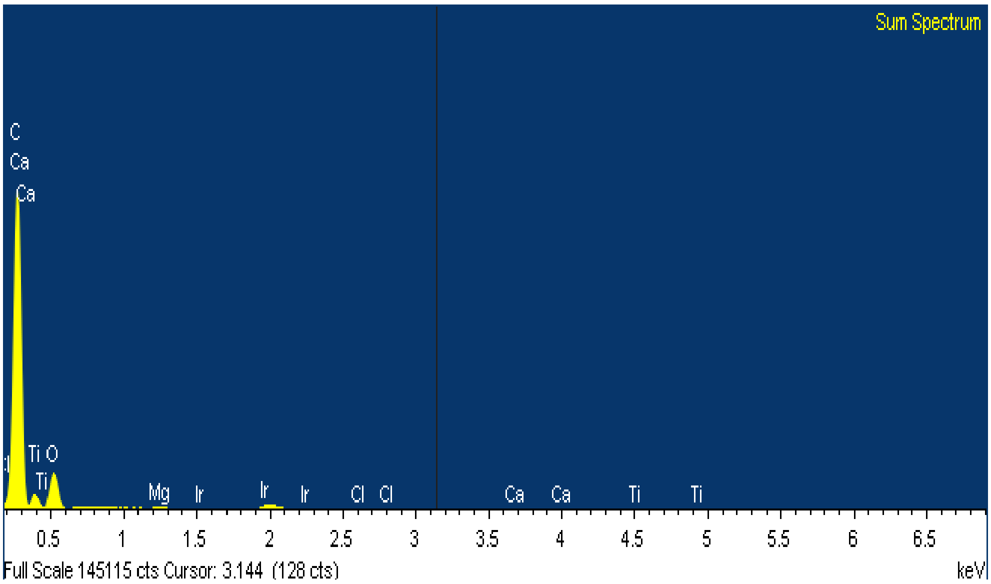
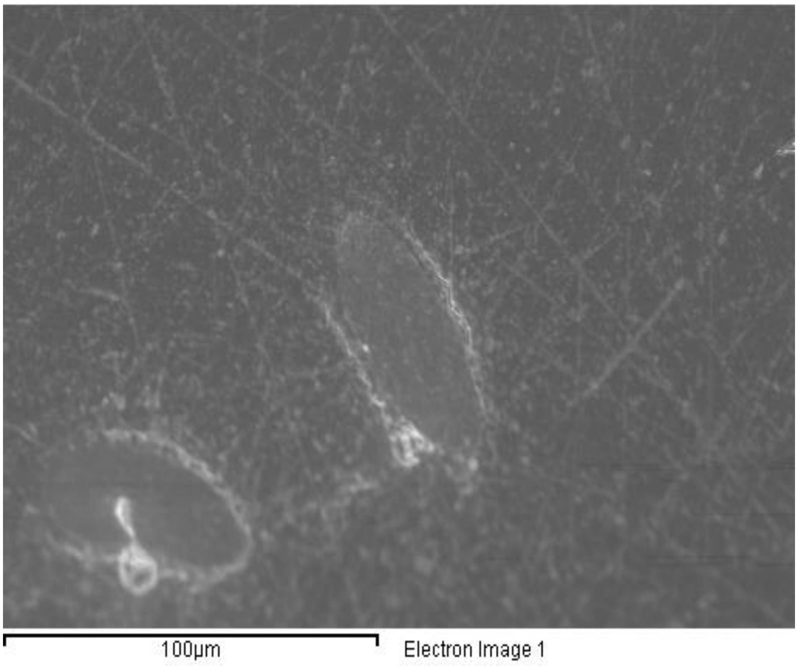


Figure A.6 The SEM photo of the cross section of a fiber and its elements spectrum. (pH = 7)



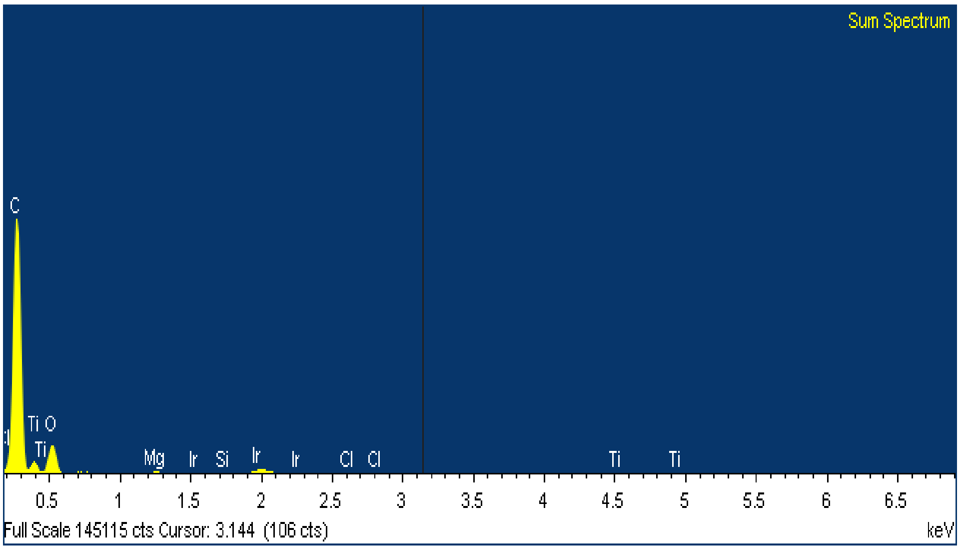
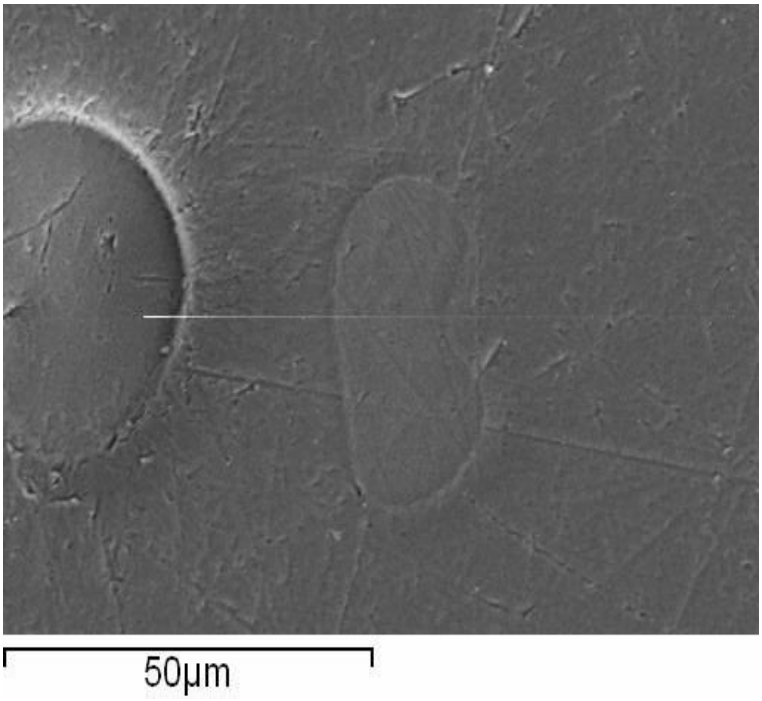


Figure A.7 The SEM photo of the cross section of a fiber and its elements spectrum. (pH = 7)



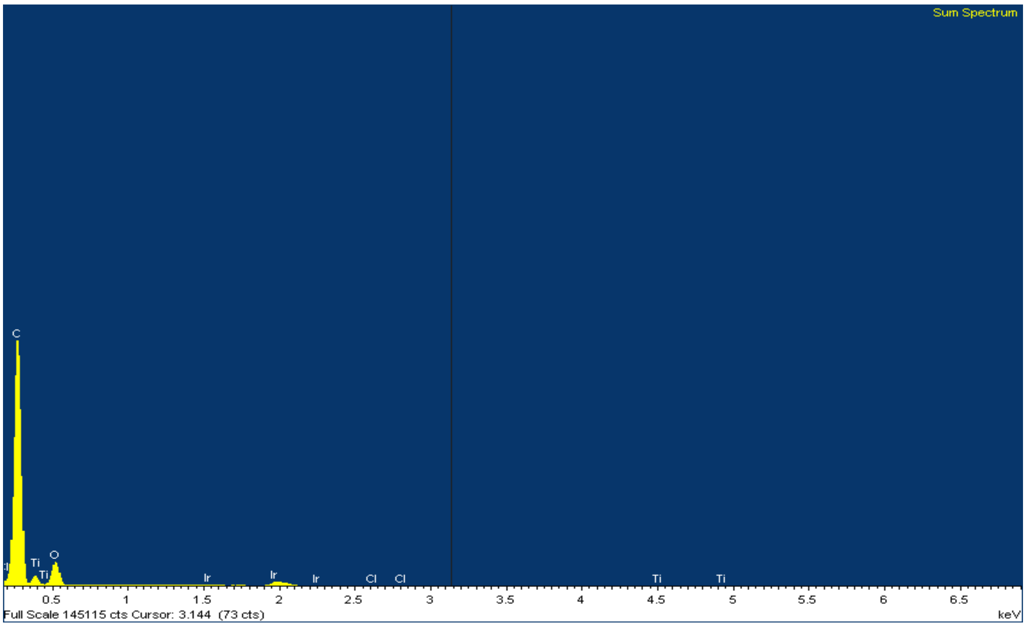
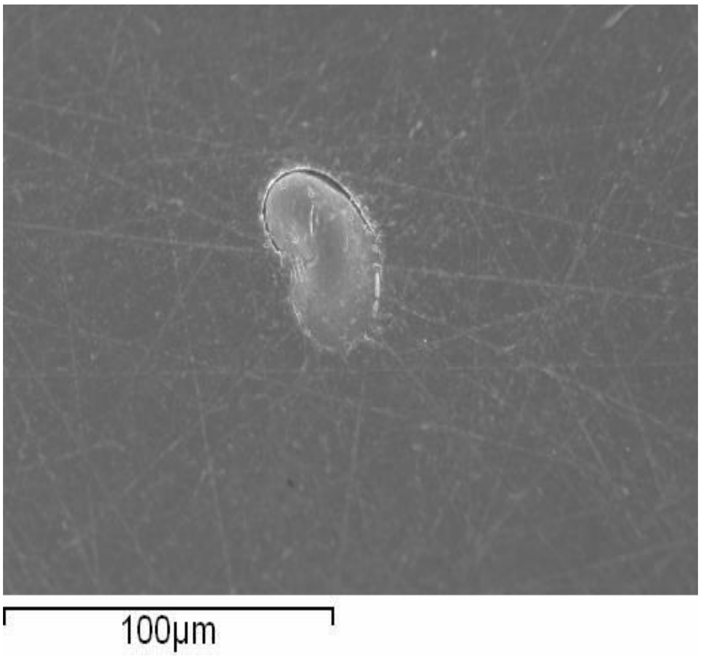


Figure A.8 The SEM photo of the cross section of a fiber and its elements spectrum. (pH = 5)



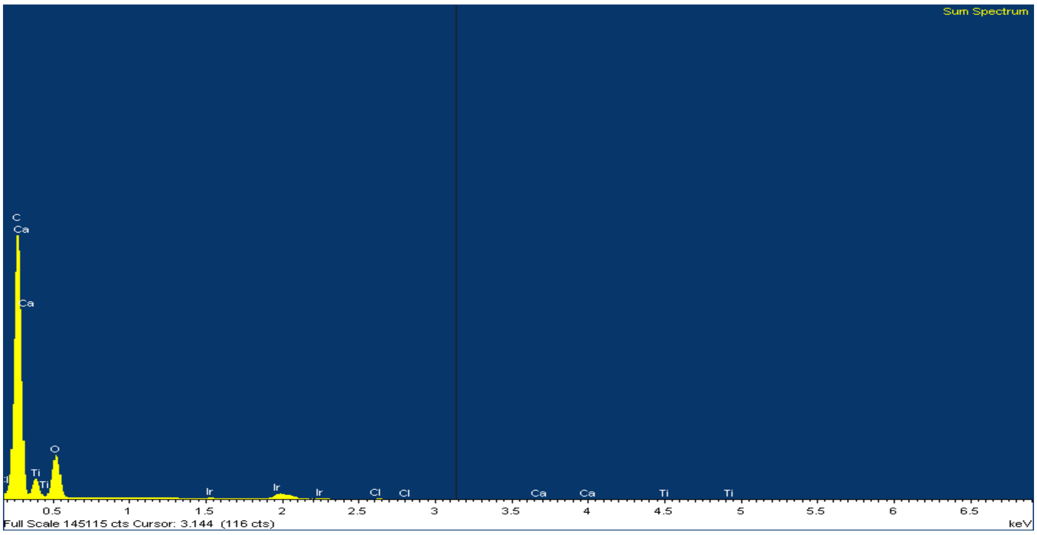


Figure A.9 The SEM photo of the cross section of a fiber and its elements spectrum. (pH = 5)

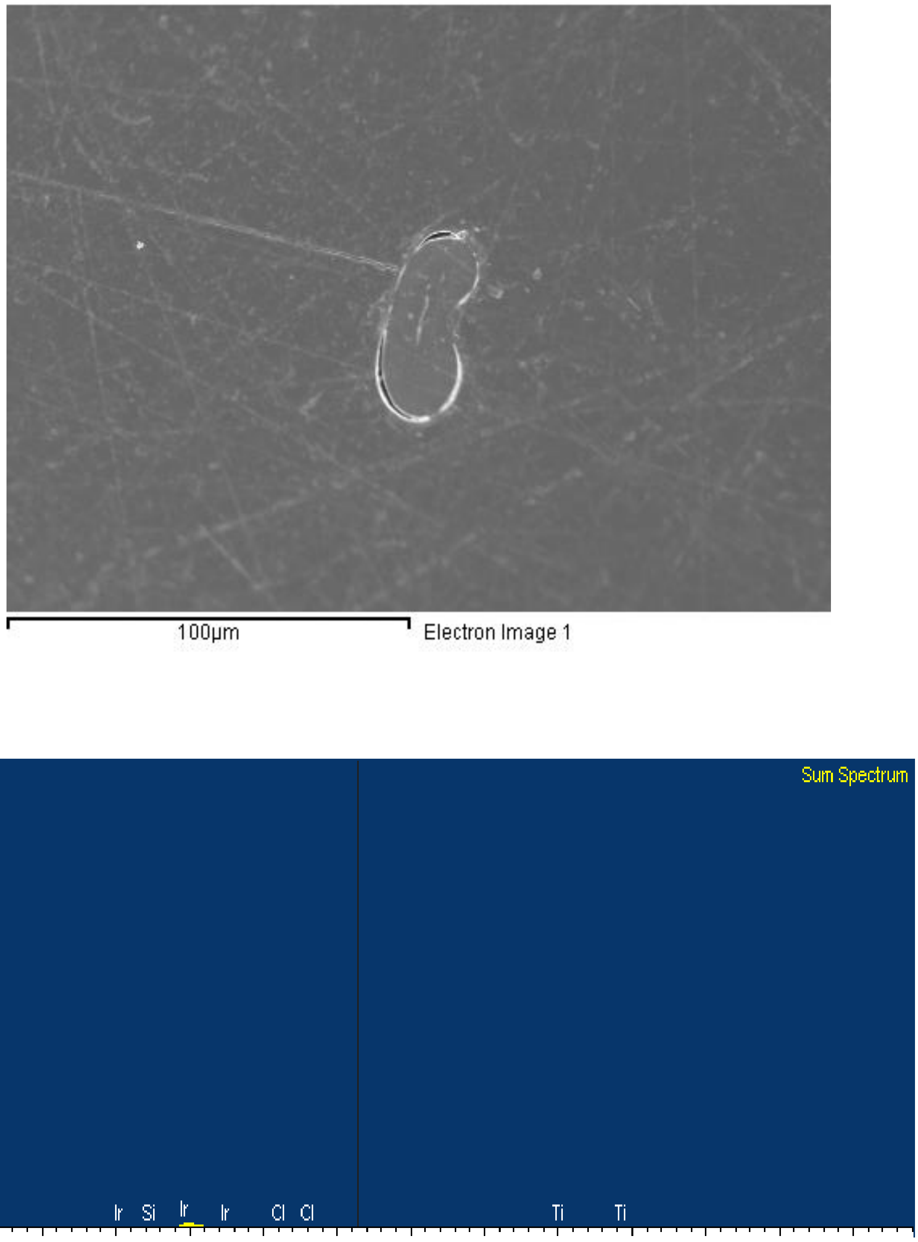
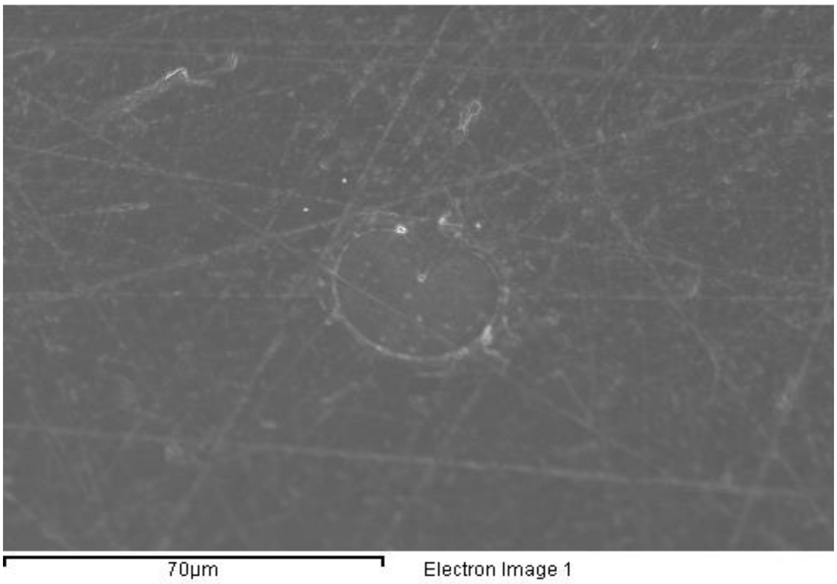


Figure A.10 The SEM photo of the cross section of a fiber and its elements spectrum. (pH = 5)



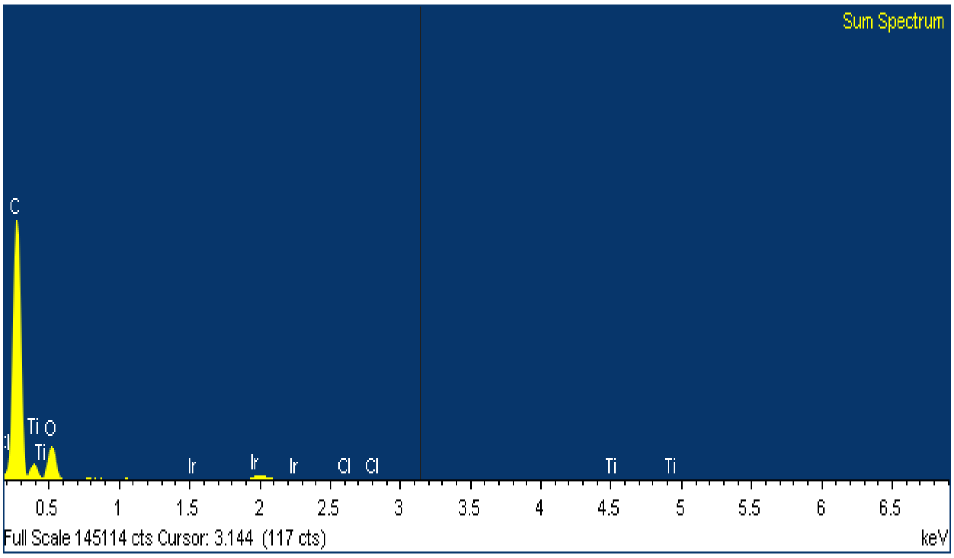


Figure A.11 The SEM photo of the cross section of a fiber and its elements spectrum. (pH = 5)

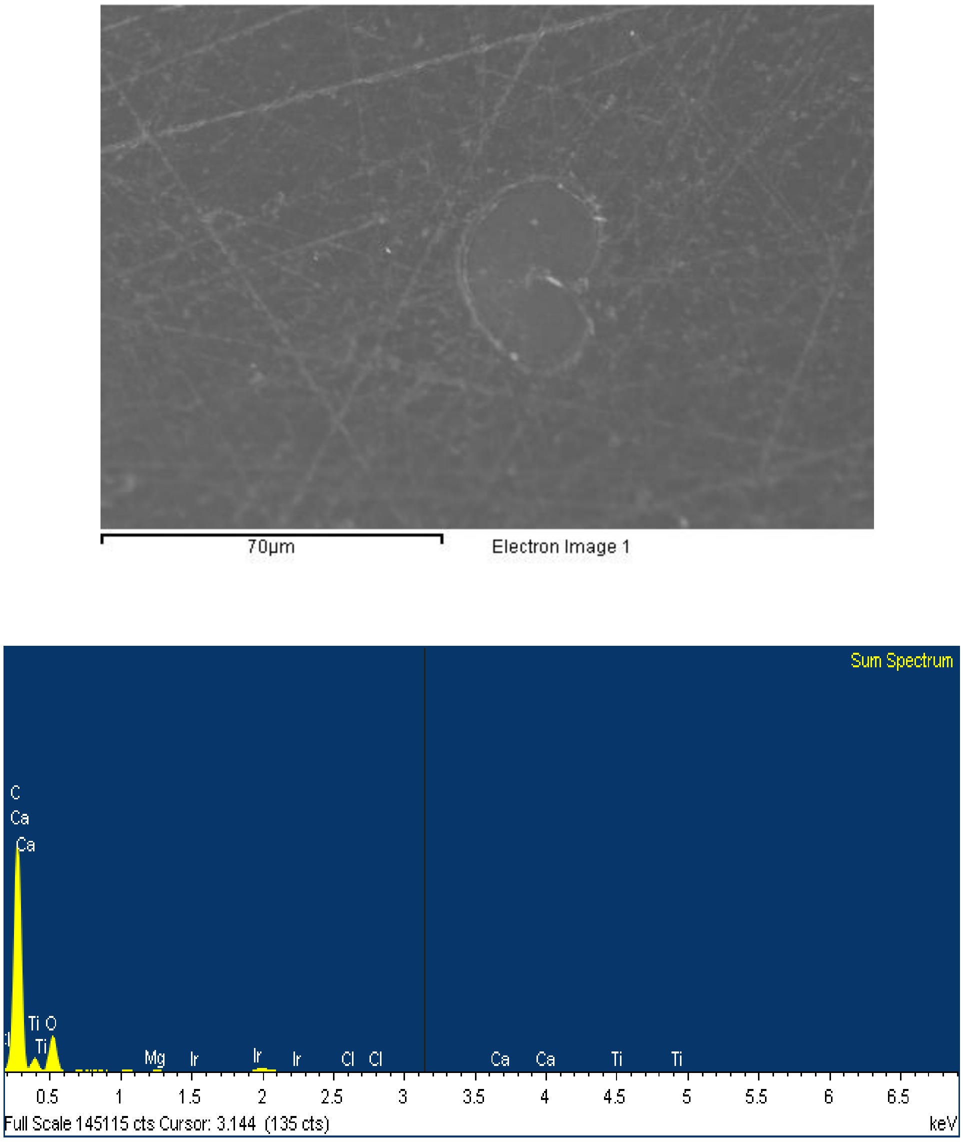
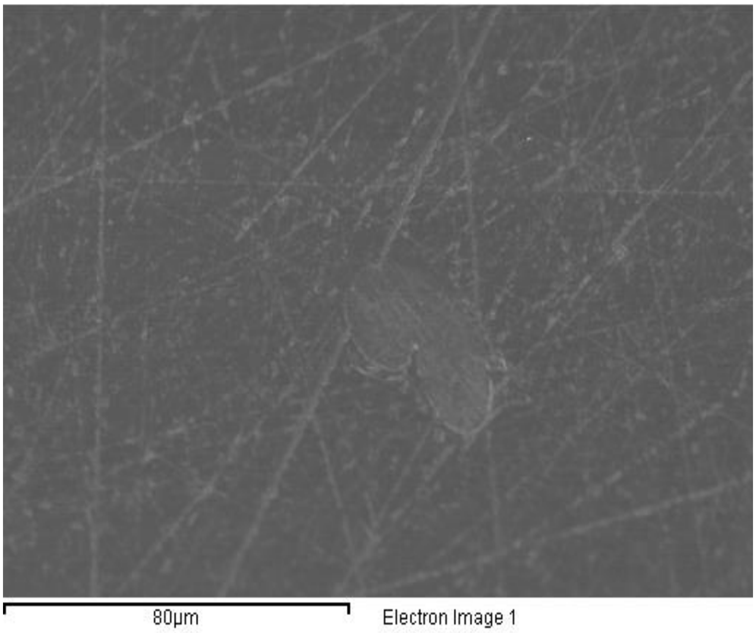


Figure A.12 The SEM photo of the cross section of a fiber and its elements spectrum. (pH = 5)



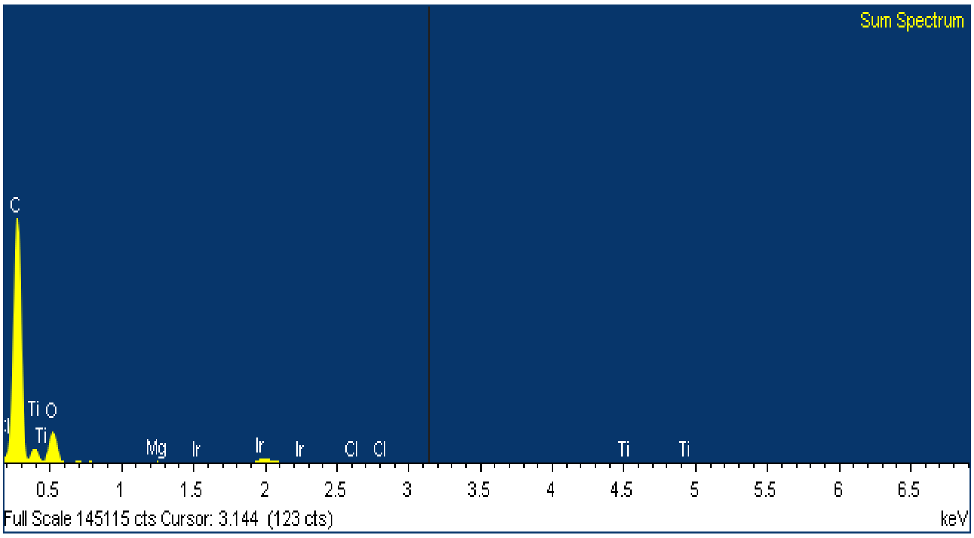


Figure A.13 The SEM photo of the cross section of a fiber and its elements spectrum. (pH = 5)

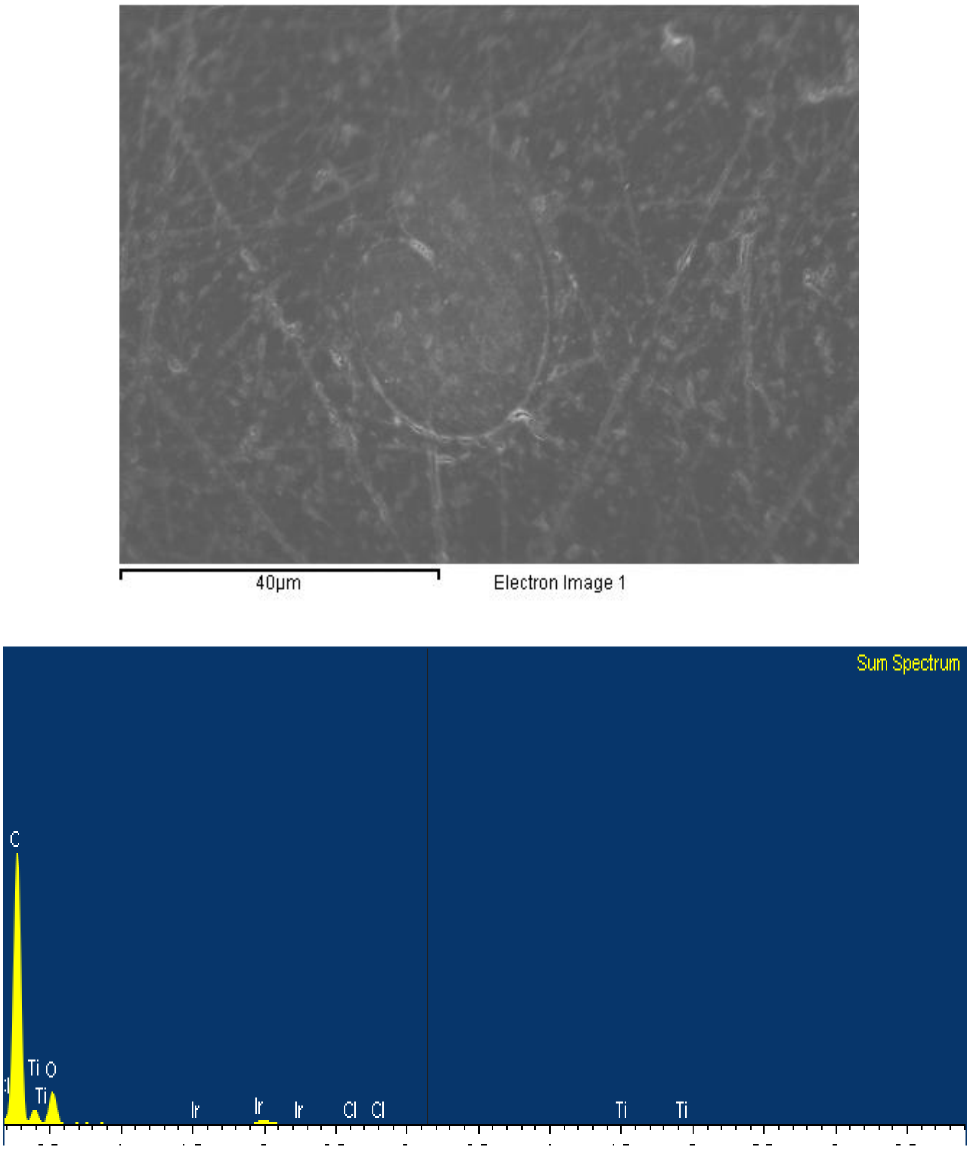


Figure A.14 The SEM photo of the cross section of a fiber and its elements spectrum. (pH = 5)

VITA

I was born on Jan 14, 1988 in China and completed the Bachalor Degree in East China University of Science and Technology majored in Bioengineering from 2006 to 2010 in China. In 2007, I was awarded the University Scholarship. After Bachalor graduation, I worked in the Waste Gas Testing Center of Zibo Environmental Protection Bureau for intenship for two months. From 2010 to 2012 I finished my Master of Science degree in Environmental Engineering in Lehigh University and then the Environmental Engineering Ph.D. program in the Department of Civil and Environmental Engineering at Lehigh University. During my Ph.D. studies I worked with Dr. Derick G. Brown and Dr. Arup K. SenGupta. I attended conferences of ACS 2015 with poster “Improved Stability of Methane-Producing Anaerobic Biological Reactors Through Novel Use of Ion-Exchange Fibers”. In addition, I also worked as a teaching assistant in CEE 170 in 2015 and 2016.